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ORIGINAL ARTICLES

CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

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INTRODUCTION

THE human body is a biochemical being. Like all living beings, it is not at a standstill; it composes and decomposes, it secretes and excretes, and as soon as it ceases to undergo changes, it is dead. In spite of the constant changes, however, the body retains a certain equilibrium, within wide limits, during health, and a disturbance of the equilibrium outside of the normal limits invariably means disease.

At times a disturbance of bodily equilibrium may be detected easily. At other times, however, both clinical judgment and laboratory technic have to be brought into play in order to establish a diagnosis of the disease.

Of late, a great number of tests have been proposed for various diseases. Some of them are easily applicable, others are not. All tests, however, have to be interpreted correctly; otherwise they are not only of no help, but may even be misleading.

Unfortunately some physicians, recent graduates as well as old practitioners, are not familiar with the interpretation of tests. Laboratory workers are constantly being asked by physicians for "complete blood" or "complete laboratory work," and on furnishing the complete report they are again asked to interpret their results. On the other hand, there is another extreme toward which some physicians, especially young graduates, are tending, namely, to place laboratory tests above all clinical signs and symptoms. The danger of such an attitude becomes evident when a special laboratory test, such as a throat culture, proves negative. We all know how frequently it happens that a culture in a case of diphtheria in spite of all clinical signs pointing to a diphtheria, is negative.

What is true of the interpretation of laboratory work in general is doubly true of laboratory work in pediatrics, for the result of a test that indicates pathology in the adult may be of no significance in the child. Such, for instance, is the case with leucocytosis and lymphocytosis which is a part of the normal blood picture in children.

Laboratory procedures in children present one more difficulty in that it often requires special methods to obtain a specimen from the patient. Such is the case with blood where the jugular vein or longitudinal sinus have to be resorted to in order to get sufficient material for a Wassermann test or for blood chemistry. Such is the case in cerebrospinal fluid where ventricular or cistern punctures have to be employed in order to obtain fluid or to inject serum. The same is even true with urine where special methods have to be resorted to in order to collect a specimen for examination.

In view of the above considerations, it has occurred to me that a course discussing both the value and limitations of laboratory tests in their relation to clinical pediatrics would meet a vital need in the curriculum of the medical school. I was fortunate enough to be able to present such a course to several classes during the last few years at the Northwestern University Medical School.

The course, as I give it, deals with the interpretation of laboratory tests as applied to pediatric practice. It does not aim to teach the student how to perform the tests. It is taken for granted that he has learned the technic during his freshman and sophomore years. The course only teaches the student the interpretation of the most important tests and their application in pediatrics. In studying the blood, the value and limitations of leucocytosis, of relative lymphocytosis, of blood sugar, nonprotein nitrogen, creatinine and urea, of alkali reserve, Wassermann, blood culture and blood grouping are discussed. In studying the urine, the value and limitations of albuminuria, reduction tests, cells, casts, quantitative chlorides, total nitrogen, urea, uric acid, ammonia and kidney function tests, are discussed. Cerebrospinal fluid, milk, stool, and pleural exudate, are studied in the same way. The interpretation of skin tests and of x-ray are also discussed.

A case is presented at every lecture and the tests previously done are interpreted and compared with the clinical findings. Occasionally, difficulty arises as to what value should be attached to certain laboratory results. It is a well-known fact that various workers attach different values to certain tests. Such, for instance, is the case with indican and creatinine in urine, and the fragility of corpuscles in the blood. In such cases I make it a point to tell the students that there is a diversity of opinion as to the value of the test, and that it is on the clinical findings, coupled with the laboratory tests, that a decision should be made.

Although it is my policy not to discuss technic with the students, I find it advisable occasionally to demonstrate or to discuss briefly certain methods of obtaining the specimen. This is usually done in cases that call for special methods on account of the age of the patient, such, for instance, as the method of obtaining blood or urine from infants.

As the course gained popularity it became necessary to give the students some references on the interpretation of the various tests and on methods of obtaining specimens in children. Surprisingly enough, this has become a tremendous task, for the standard text books on pediatrics or those on laboratory methods do not always present the most practical methods of obtaining specimens from children or the clinical application of the tests in children.

It was the necessity of finding a text, and the pressure brought on me by my students and by a number of teachers of pediatrics that called forth the following series of articles which is to be published in the *Journal of Laboratory and Clinical Medicine*. In this outline, I have endeavored to correlate the laboratory tests with clinical pediatrics, with especial emphasis on pediatric interpretation.

The series of articles will consist of the following:

1. Methods of clinical procedures in infants and children. This will include: physical examination, removal of blood from the jugular vein and from the longitudinal sinus; spinal, ventricular and cistern puncture; collection of urine; lavage and gavage; skin tests, etc.

The methods are simplified to such an extent that they can be done by every practitioner of medicine.

2. A description of simple laboratory tests that can be carried out in the ordinary laboratory or even office.

3. Above all, a discussion will be given of the interpretation of various clinical and laboratory tests as applied to infants and children.

The purpose of the various tests, and the clinical application of the results of the tests to infants and children will be discussed. Whenever necessary, emphasis will be laid on the difference between the normal standards in children and those in adults.

The articles are written, not for the pediatrician or trained laboratory worker, but for the medical student and for the general practitioner. An endeavor has been made throughout to be simple rather than complex, practical rather than theoretical.

CASE HISTORY

In the case of a very small child the history must naturally be obtained from the parent. In the case of an older child the history should be taken both from the parent and the child. Many a child will describe its symptoms more vividly than its mother, who usually adds some flavor to the story. If possible, the mother should be heard in the absence of the child, so that the child will not be influenced by her story. The history should include the following:

Family History.—This should consist of questions as to the number of children in the family, their ages, and the state of their health; as to the state of the mother's health during the last pregnancy, as to miscarriages preceding or following the birth of the patient; as to the incidence of disease in family with special reference to tuberculosis, syphilis, rheumatism, heart

disease, alcoholism, epilepsy, idiocy and neurosis. All of the above diseases are often familial in character and may have a direct or indirect bearing on the present condition of the patient.

PERSONAL HISTORY.—This should include the following questions:

1. *Birth.*—Whether the patient was born at full term or was premature, the latter of which may explain congenital weakness or rickets, whether the birth was natural or instrumental, whether the labor was precipitate or prolonged. Modern research has shown that instrumental or prolonged labor may be responsible for brain hemorrhage in the newly born, which may later manifest itself in a spastic paralysis.

2. *Method of Feeding.*—Whether the child is or was breast-fed and for how long a period. If artificially fed, inquiry should be made as to the kind of food and the frequency of feeding, also as to whether the child was or is getting orange juice or other fruit juices. The method of feeding may be responsible for malnutrition, scurvy, tetany, and intestinal disturbances.

3. *Development.*—Inquiry should be made as to the time the child first sat up, walked, and talked, an important consideration in the diagnosis of rickets, idiocy, and congenital syphilis. Inquiry should also be made as to the time of the eruption of the first tooth and of the subsequent teeth. The latter, however, is not nearly so important in diagnosis as was formerly thought to be; while many rachitic children erupt their teeth late, there are many healthy children in whom the first tooth is not erupted before 12 or 15 months of age. On the other hand, some congenitally weak children erupt their teeth early.

PREVIOUS DISEASES.—The history of the following diseases is of the utmost importance:

1. *Intestinal Disturbances,* which may account for malnutrition.

2. *Rickets,* which may be responsible for deformities of the chest or for delayed development.

3. *Tetany,* which may be responsible for convulsions or other neurotic manifestations.

4. *Scarlet Fever,* because of the fact that one attack usually confers immunity, and because of the frequency of nephritis, otitis and adenitis following the disease.

5. *Measles,* which often predisposes the patient to bronchopneumonia and otitis media.

6. *Pertussis,* which is nearly always followed by chronic bronchitis and is often followed by pneumonia and encephalitis. It also predisposes the patient to tuberculosis of the respiratory tract.

7. *Diphtheria,* which may be responsible for myocarditis, stenosis of the larynx and paralysis of various parts of the body.

8. *Tonsillitis and rheumatism,* which may be responsible for chorea and carditis. An effort should be made to inquire as to the presence of the so-called "growing pain," which is often nothing more or less than an acute or subacute articular rheumatism.

9. *Pneumonia,* which makes a second attack more liable, and which is often followed by complications.

10. *Meningitis and encephalitis* which may be responsible for many nervous disturbances, such as convulsions or epilepsy.

11. *Trauma,* which may be responsible for a multitude of affections.

12. *Surgical diseases,* which may cause adhesions or leave other sequela.

It should be kept in mind that there is hardly a disease which does not leave its trace upon the child's body or mind.

PRESENT HISTORY.—Careful attention should be paid to the onset of the present disease, whether it was sudden or slow, whether accompanied by fever, chills, convulsions, vomiting, diarrhea, constipation, cough, sneezing or running of the eyes.

Fig. 1.—Method of examination of infant's throat.

Fig. 2.—Method of holding infant for examination of chest.

The mode of onset may at once throw light on the nature of the disease. It may for instance, help decide between measles and scarlet fever. It is well known that scarlet fever sets in suddenly while measles comes on gradually with coryza, conjunctivitis and cough. Vomiting is a cardinal symptom of scarlet fever, although it may be present in other diseases and occasionally is absent in scarlet fever. Convulsions are rather frequent at the onset of infectious diseases in children, and therefore do not necessarily signify an organic disease of the nervous system; fever is present in many diseases, yet the height and type of fever may help in the diagnosis.

PHYSICAL EXAMINATION

The patient should be completely undressed, no matter what part of the body is affected.

POSITION OF PATIENT.—The position of the child during examination de-

pends a great deal on the age of the child and on the part of the body to be examined.

As a rule, there is no difficulty in examining older children as they can be maneuvered easily to whichever position is desired. The examination

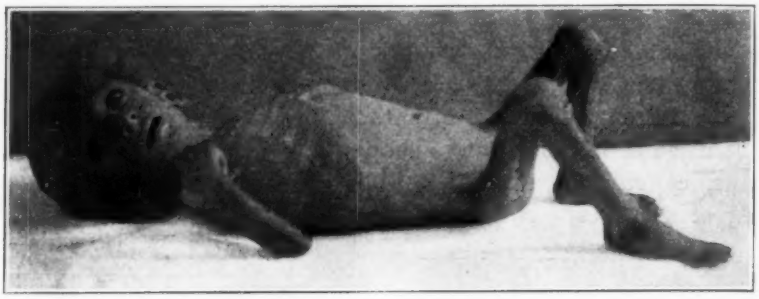

Fig. 3.—General appearance of extremely emaciated infant, one year of age.

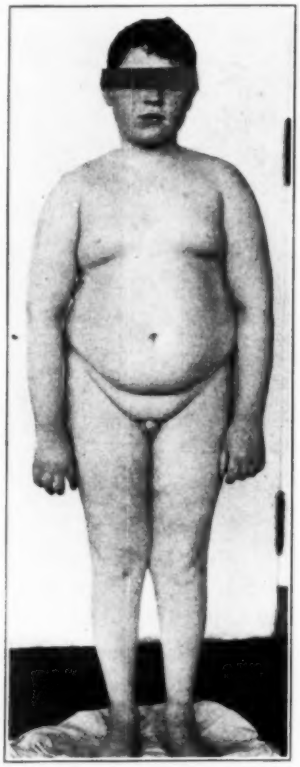

Fig. 4.—Boy of ten presenting Fröhlich's syndrome.

of infants, however, is often beset with difficulties. It is therefore important to select the best position for the purpose of examination. Some physicians prefer to examine infants in the recumbent posture on a table; others prefer to have the infant in recumbent posture on the mother's lap; and still others prefer to have the infant in a sitting position on a table or on the

mother's lap. I believe that the examination of an infant is best accomplished by having the mother or nurse sit on an ordinary kitchen chair and hold the infant on her lap either in the recumbent or in the sitting position, the sitting posture being preferable.

The various parts of the body require different positions on the part of the child. The anterior portion of an infant's chest is best examined by having the mother or nurse hold the baby's legs tight in her lap causing the baby's occiput to lean against her chest and restraining the baby's head with one hand and his arms with her other hand. The same position should be used for the examination of the mouth and throat (Fig. 1).

Fig. 5.—Characteristic posture of patient with decompensated heart.

Fig. 6.—Orthopnea in patient with decompensated heart.

The posterior portion of the infant's chest is best examined by placing the infant's arms around the mother's or nurse's neck (Fig. 2).

To examine an infant's ears, the baby should be placed opposite the light in such a way that one cheek will be leaning on the mother's chest.

NOURISHMENT.—The general appearance of the patient will show at a glance whether he is well developed, underdeveloped (Fig. 3) or overdeveloped (Fig. 4). More accurate information can be obtained by weighing and measuring the patient, especially in the case of an infant. In the latter, the weight is one of the best indications as to whether or not the feeding agrees with the patient. In interpreting the weight of the patient, however,

the birth weight, the stature and the racial characteristics should be taken into account. It is natural that what is good weight at three months for a prematurely born infant would be poor weight for a full term baby.

EXPRESSION.—A normal child is happy and lively. An idiotic child stares; a child with typhoid is apathetic; with alimentary intoxication is toxic; with meningitis or pneumonia, has a "pinched" expression.

POSTURE.—A child suffering from pneumonia usually lies on the affected side; one with peritonitis, lies on his back with his legs drawn up; a patient with cardiac decompensation has to be propped up on pillows (Fig. 5); and in the very advanced stages assumes the orthopneic position (Fig. 6). A patient suffering from asthma has to sit up during an attack. The sleeping posture is also important. Children suffering from adenoids dig

Fig. 7.—Wrinkling of skin in infant suffering from congenital syphilis.

their head into the pillow. Rickety children move from place to place in their sleep and rub their occiput against the pillow.

SKIN.—Observation should be made as to the color of the skin, the presence of anemia and jaundice, as to the presence of eczema, urticaria, exanthematous rashes, wrinkling such as is present in congenital syphilis (Fig. 7) scaling, as in overlooked scarlet fever, ichthyosis, and certain local applications, enlarged glands, birthmarks, scars, new growths and edema.

HEAD.—The shape of the head, the patency of the cranial sutures, the size and patency of the fontanelles, the presence of craniotabes and parietal bosses, and amount and type of hair and various deformities should be looked for.

The shape of the head may disclose the presence of a microcephalus,

which invariably signifies idiocy; hydrocephalus which is secondary to many organic brain affections (Fig. 8), or square head seen in rickets.

The cranial sutures are open the first week or two of life. After that if open, they signify prematurity, rickets or syphilis.

A normal fontanelle should be on a level with the rest of the skull. A distended fontanelle usually signifies increased intracranial pressure. A depressed fontanelle often signifies extreme weakness. The time of closure of the fontanelles is also important. The posterior fontanelle closes normally between one to three months of age and the anterior fontanelle at sixteen to eighteen months of age. A delay in closure signifies rickets, malnutrition or hydrocephalus.

Fig. 8.—Marked hydrocephalus.

Eyes.—The expression of the eyes, the width of the palpebral fissures, the length of the eyebrows, the size and equality of the pupils, and their reaction to light and accommodation should be observed. Strabismus, nystagmus, exophthalmus, corneal ulcer, interstitial keratitis, conjunctivitis, ptosis and deformities should be looked for.

The expression of a child's eye will tell at a glance whether the child is active or drowsy, whether he is mentally sound or deficient. An acute observer can often even get an impression of the type of disease by the expression of the eyes.

The width of the palpebral fissure may disclose the presence of a facial paralysis, of an exophthalmus and of mongolian idiocy.

Ptosis is due to paralysis of the third cranial nerve. It is present in

encephalitis, acute anterior poliomyelitis and meningitis. Investigation should be made as to the cause.

Long eyelashes are considered by some observers to signify latent tuberculosis or phthisical habit in children. Racial characteristics must, however, be taken into consideration. No conclusions should therefore be drawn from the mere length of the eyelashes. Markedly dilated or markedly contracted pupils may be due to intracranial pressure. They may, however, be due to the effects of drugs such as the dilation of the pupils by atropine and the contraction of the pupils by opiates.

Unequality of pupils invariably means organic nervous disturbances. The same is true with alteration in the reaction of the pupils to light and accommodation. Strabismus is present in many infants during the first year of life. The condition usually corrects itself. In older children strabismus requires interference either by refraction or operation.

Nystagmus always means organic disturbance.

Exophthalmus is seldom seen below the age of ten, and when present it requires thorough investigation.

Interstitial keratitis should make one suspect congenital syphilis tarda. Acute conjunctivitis is present in measles and in grippe. It may also be due to other infections and to trauma. The etiology of phlyctenular conjunctivitis is still a disputed question. Some claim it to be due to tuberculosis. The presence of phlyctenular conjunctivitis should therefore make one investigate the case further.

Trachoma is rather infrequent in American children but should be looked for. Complete absence of vision is due to a variety of causes, amaurotic family idiocy being one of them.

Ears.—The shape of the external ear should be observed as it may give clew to the diagnosis of idiocy. Discharge of middle ear and swelling of mastoid should be looked for. Ear drums should be examined by reflected light for redness and bulging. The latter often solves the diagnosis of an obscure fever.

Nose.—The shape of the nose, deformities and nasal obstructions should be noticed. The shape of the nose often serves as a clew in the diagnosis of congenital syphilis; nasal obstruction is frequent in children suffering from adenoids, sinus infection and general grippe. Discharge from the nose may be due to a mere cold, but may be diphtheritic in character. Every mucopurulent discharge from the nose should therefore be considered suspicious of diphtheria and should be cultured for Klebs-Loeffler bacilli.

In infants, a chronic discharge from the nose should also be suspected of syphilitic snuffles.

Mouth.—The color of the lips should be noted for cyanosis and anemia, for herpes labialis, frequent in pneumonia and meningitis, and for fissures frequent in congenital syphilis.

The gums should be examined for their color, for hemorrhage present in scurvy and in infections of the mouth, and for abscesses.

The presence or absence of teeth should be observed; delayed dentition, decayed teeth and irregular teeth should be noticed.

Delayed dentition is frequent in rickets, although no diagnosis of rickets should be made on mere delayed dentition. Decayed teeth are frequently the source of generalized infection and should therefore be noted carefully. Irregular teeth may be present in rickets and a number of other conditions of malnutrition. Hutchinson's teeth are one of the signs of late congenital syphilis.

The mucous membrane of the mouth should be examined for thrush, stomatitis, and enanthema. It is well known that all exanthema also have an enanthem. Of special diagnostic importance is the presence of Koplik's spots preceding the exanthem of measles.

The tongue should be examined for changes in color. A coated tongue is present in most infectious diseases, strawberry tongue is one of the cardinal symptoms of scarlet fever. The presence of ulcers, cysts, and scars should also be noted; the latter is often present in epileptic patients.

The hard and soft palate should be noted for enanthema, for clefts and other deformities.

The tonsils should be noted for their size, for exudates and abscess. The rest of the pharynx should also be noted for inflammations, exudates and edema. Every suspicious throat necessitates a throat culture, every edematous pharynx should be examined further by palpation with index finger for the presence of a retropharyngeal abscess.

Neck.—The neck should be observed for enlargements of cervical glands, present in acute and chronic infections, for the size of the thyroid, for rigidity such as is present in meningitis and meningism, for head-drop which is one of the diagnostic symptoms of an acute anterior poliomyelitis and for anomalies such as thyroglossal cysts.

Chest.—The expansion of the chest and the presence of asymmetry should be noted. Asymmetry is usually present in pleural effusion and cardiac affections. Distended veins over the chest should also be noted as their presence usually indicates an obstruction of the mediastinum by tumor or tuberculous glands, rosary, Harrison's groove occurring in rickets, and retraction of the lower part of the sternum, as occurs in advanced laryngeal diphtheria, should be looked for.

Heart and Lungs.—Palpation, percussion and auscultation should be carried out the same as in adults. Percussion should be very light. The heart should be outlined and the heart tones noted. It should be remembered that the left heart border in infants is usually outside the nipple line and that the heart tones in infants and in children are louder than in adults. No murmur should be considered "mere functional" unless proved to be so by prolonged observation.

In normal infants the percussion note over the lung is higher pitched than in adults except over the right upper lobe posteriorly. The breath sounds are also louder in pitch than in adults. Any change in percussion and auscultation of the chest should be carefully noted.

ABDOMEN.—The shape of the abdomen should be observed for edema, present in decompensated hearts, in inflammations and new growths of liver, and kidneys; for typanites, present in peritonitis and in many infectious diseases and for retraction, present in miliary and other forms of tuberculosis, also in wasting due to prolonged illness. The abdomen should be palpated for areas of tenderness and rigidity. In infants with severe vomiting reverse peristalsis should be looked for.

Appendicitis in infants and children does not produce the marked rigidity observed in adults. Absence of rigidity, therefore, does not exclude appendicitis. On the other hand pneumonia is frequently accompanied by marked rigidity. Not every rigid abdomen therefore is an operative case.

Fig. 9.—Abdomen in case of decompensated heart.

The liver and spleen should also be palpated for enlargement and tenderness. Enlargement of the liver accompanies decompensated heart, hereditary syphilis and new growths. Enlargement of spleen is frequent in syphilis, leukemia, decompensated heart and acute infectious diseases, especially malaria and typhoid. It is also found in many cases of rickets.

GENITO-URINARY ORGANS.—Phimosis and undescended testicles in the male, and vaginitis in the female should be looked for. Hernia should also be looked for.

SPINE.—Tender spots and deformities, such as kyphosis, lordosis and scoliosis, should be looked for.

EXTREMITIES.—General development, epiphyseal enlargement, such as is

present in rickets; tenderness, present in the early stages of acute poliomyelitis, in grippe, and in scurvy; edema and desquamation should be observed.

NERVOUS SYSTEM.—General attitude of patient, type of cry, mentality, choreiform movements, exaggerated or absent reflexes, should be observed.

OTHER EXAMINATIONS.—To complete the examination, the temperature should be taken, the urine and blood should be examined, and a tuberculin test should be made. In some cases, a nose and throat culture should be taken. All of these examinations will be discussed under their respective headings.

(To be continued.)

MIXED TUMORS OF THE UTERUS*

BY A. J. PETERSEN, M.D., CHICAGO, ILL.

MIXED tumors of the uterus contain a variety of mesoblastic tissues such as smooth and striated muscle, fibrous connective tissue, fat, bone, cartilage, endothelial tissue and certain undifferentiated tissues derived from the mesoderm. Wilms' monograph in 1900 contains a review of the mixed tumors of the uterus reported in the literature at that time and explains clearly their origin by displacement of embryonal mesoblastic tissue rests along the course of the Wolffian duct. Since Wilms' monograph many other mixed tumors of the uterus have been reported. A summary of fifty of these reports demonstrates that twenty-seven occurred in the fundus of the uterus, the others in the cervix; thirty-two contained cartilage, four bone, fourteen smooth muscle, eighteen striated muscle, five fat, two endothelium, three carcinoma, and almost all sarcoma tissue.

Probably the most characteristic feature of these tumors is their histological structure, that is, their content in a variety of mesoblastic tissues. Almost all of the mixed tumors reported are regarded malignant, although benign tumors are recorded (Perlstein). This malignancy is manifested by a local recurrence after removal rather than by the appearance of remote metastases. The metastases, while infrequent and late, usually do not contain heterogeneous tissues, and occasionally contain tissues not found in the primary tumor. Twenty-eight per cent of the reports of mixed tumors reviewed mention metastases, most of which were confined to the abdomen and pelvis. Tumors of the fundus seem to infiltrate the pelvic tissues later than those of the cervix. Mixed tumors of the uterus have been reported for ages ranging from two years to seventy-five years, although 50 per cent have occurred in women over fifty years of age. Uterine activity seems to have no relationship, for mixed tumors have been found in nulliparous women as often as in multiparous. Clinically they are not easily differ-

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entiated from other malignant tumors of the uterus. Bleeding or leucorrhea is common and pelvic pain is inconstant. Large tumors by pressure may cause symptoms referable to the urinary bladder, or to venous obstruction. Necrotic fragments of the tumor sometimes break away and escape through the vagina. Cachexia, anemia and emaciation appear in the later stages of the disease.

TABLE 1*

AUTHOR	PLACE OF PUBLICATION	DIAGNOSIS
1. Lobstein	See Meckel, J. F., Handbuch d. path. Anatomie, ii, 311., Leipz., Reclam, 1816.	Lipoma; no microscopic examination
2. Seegar	See Knox, J. H., Bull. Johns Hopkins Hosp. 1901, xii, 318.	Lipoma; no microscopic examination
3. Lebert, H.	Traité d'anat. Pathol. gen. et spec. Atlas., Tom. I, Pl. 16, Fig. 11. Paris, Bailliére et fils, 1857.	Lipoma; no microscopic examination
4. Smith, T.	Tr. Path. Soc., Lond. 1861, xii, 148. See Williamson, H. and Brockman, R. St. L., Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec. 1920, xiii, 136.	Fibromyolipoma; microscopic examination in 1920
5. Stroinski, O.	Chicago Med. Review, 1880, ii, 469.	Lipoma
6. Orth, J.	Lehrbuch d. spec. path. Anatomie, ii, 485. Berlin, Hirschwald, 1893.	Lipoma; no microscopic examination
7. Kelly, H. A. 8. and Cullen, T. S.	Myomata of Uterus, p. 162, Phila. and Lond., Saunders Co., 1909.	Lipomyoma (2)
9. Brünings	Verhandl. d. deutsch. Gesellsch. f. Gynäk. zu Berlin, 1899, viii, 348.	Lipomyoma
10. Wilms, M.	Die Mischgeschwülste, Heft 2 Leipz., Georgi, 1900.	Lipomatous areas of mixed tumor
11. Merkel, H. 12. Merkel, H.	Ziegler's Beitrage z. path. Anatomie u. z. allg. Pathologie, 1901, xxix, 507.	Lipofibromyoma Lipoma
13. Knox, J. H. M.	Bull. Johns Hopkins Hosp., 1901, xii, 318.	Lipomyoma
14. v. Franqué	Verhandl. d. deutsch. Gesellsch. f. Gynäk. zu Giessen, 1901, ix, 491.	Lipofibromyoma
15. v. Jacobson	Ztschr. f. Heilkunde, xxiii, N. F. 3, Abth. f. path. Anatomie, 1902, p. 139.	Lipofibromyoma
16. Seydel, O.	Ztschr. f. Geburtsh. u. Gynäk. 1903, l, 274.	Lipofibromyoma
17. Meyer, R.	Ztschr. f. Geburtsh. u. Gynäk. 1903, l, 274.	Lipoma
18. Pollak, E.	Wien. klin. Wchnschr. 1903, xvi, 68.	Lipofibromyoma
19. Gebhard, C.	Ztschr. f. Geburtsh. u. Gynäk. 1903, xlviii, 111.	Lipomatous areas of mixed tumor

*This table includes all of the lipomas found recorded after thorough search in the literature.

TABLE I—*Continued.*

AUTHOR	PLACE OF PUBLICATION	DIAGNOSIS
20. Spuler, R.	Centralblatt f. allg. Path. u. path. Anat. 1905, xvi, 337.	Lipomatous areas of mixed tumor
21. Ellis, A. G.	Surg., Gynec. and Obst. 1906, iii, 658.	Lipoma
22. Kauffmann, E.	Ztschr. f. Geburtsh. u. Gynäk., Berlin, 1907, lx, 312.	Lipoma
23. Walkhoff, E.	Festschr. f. Rindfleisch, Leipz., 1907, 212.	Lipomyosarcoma
24. Sitzenfrey, A.	Ztschr. f. Geburtsh. u. Gynäk., Stuttg., 1910, lxvii, 32.	Liposarcoma
25. Heddäus, A.	See Perlstein, I., Surg., Gynec. and Obst. 1919, xxviii, 43.	Lipomatous areas of mixed tumor
26. Ley, G.	Jour. Obst. and Gynec., Brit. Emp., Lond. 1914, xxv, 42.	Lipofibromyoma
27. Murray, H. L. & Littler, R. M.	Jour. Obst. and Gynec., Brit. Emp., Lond., 1914, xxv, 26.	Lipomatous areas of mixed tumor
28. Elkin, C. W. W. & Haythorn, S. R.	Surg., Gynec. and Obst. 1917, xxv, 72.	Lipoma
29. Lockyer, C.	Fibroids and Allied Tumors, p. 65, Lond., Macmillan & Co., 1918. See Williamson, H. and Brockman, R., Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec., 1920, xiii, 136.	Fibromyolipoma
30. Williamson, H. and Brockman, R. St. L.	Proc. Roy. Soc. Med., Lond., Sect. Obst. and Gynec., 1920, xiii, 136.	Myolipoma
31. Schleussner, R. C.	Proc. N. Y. Path. Soc. 1921, N. S., xxi, 33.	Lipoma

Diagnosis finally depends upon the histological examination of the tumor tissue, although the presence of cartilage or other characteristic tissue in masses large enough to recognize grossly permits a tentative diagnosis, at least. The prognosis after removal, as a rule, is unfavorable and the duration of life after the appearance of symptoms usually is one and one-half to two years. One patient is reported by Peuch and Massabuan (see Perlstein) to have lived six years.

The first of the two mixed tumors of the uterus reported here contains the variety of tissues usually described in such tumors, the second macroscopically contains so much adipose tissue that it seems grossly to be a lipoma, but in the histological preparations there are other tissues than fat cells. Table I contains a list of tumors reported in the literature as lipomas of the uterus, but where careful histological studies have been made there is reference to tissue other than fat cells and the propriety of considering them lipomas rather than mixed tumors is doubtful. However, those tumors containing large amounts of fat seem to be relatively benign, and

have been found accidentally either during an operation or by postmortem examination.

Briefly the salient features of each of the tumors reported here are as follows:

1. Occurred in the body of a uterus surgically removed from an unmarried woman, aged 60 years, with a clinical diagnosis of malignant fibromyoma (service of Dr. T. J. Watkins). The tumor is 8 cms. in diameter, the

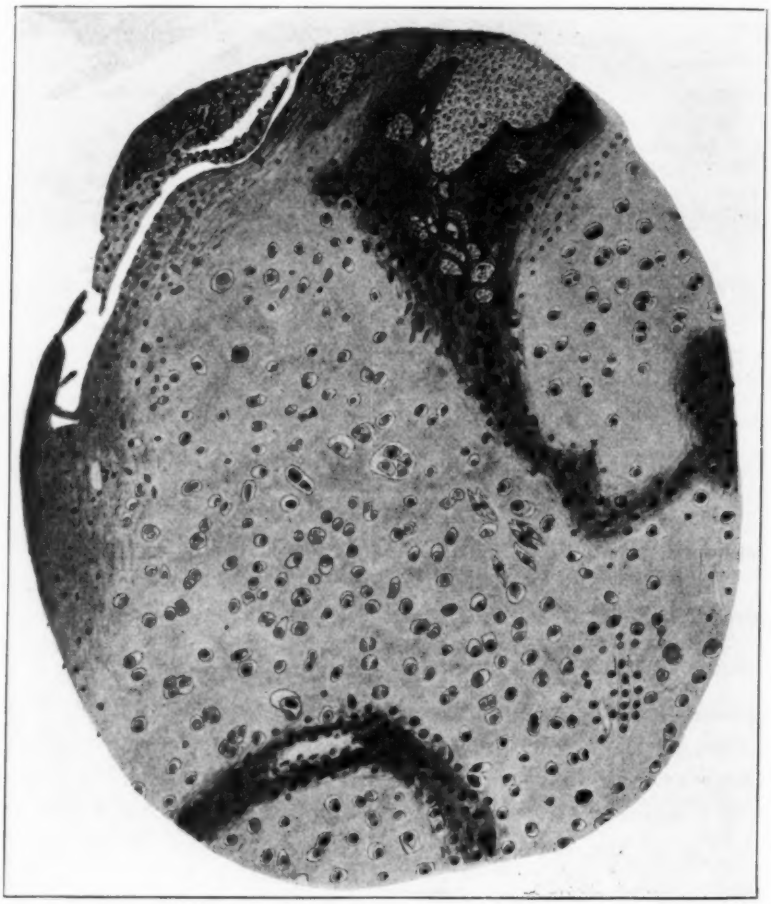

Fig. 1.—Illustrating the areas of cartilage and fibrous connective tissue in the mixed tumor of the uterus.

lining of the uterus covering it contains irregular polypi and even macroscopically in the tumor there are large masses of tissue recognized as cartilage. Microscopic preparations contain hyalin cartilage, trabeculae of bone, smooth muscle, alveoli of round and spindle-shaped cells and fibrous tissue. In the sections studied, roughly 2 per cent of the tissue is bone, 35 per cent is hyalin cartilage, 35 per cent is smooth muscle, 30 per cent is white fibrous connective tissue, and 1 per cent is alveoli of round and spindle cells. (Figs. 1 and 2.) The patient died three months after the operation

with recurrence of the tumor in the pelvis and one year after the appearance of symptoms.

2. This tumor was diagnosed clinically a fibromyoma, in an unmarried woman, aged fifty-four years, and the uterus was removed completely (service of Dr. T. J. Watkins). In its submucosa is a light yellow, oily, almost round tumor, 10 cms. in diameter, encapsulated and easily enucleated. The endometrium covering it is edematous, but otherwise it is normal. At the level of the internal os is a fibrous tissue scar of the cervical canal, markedly

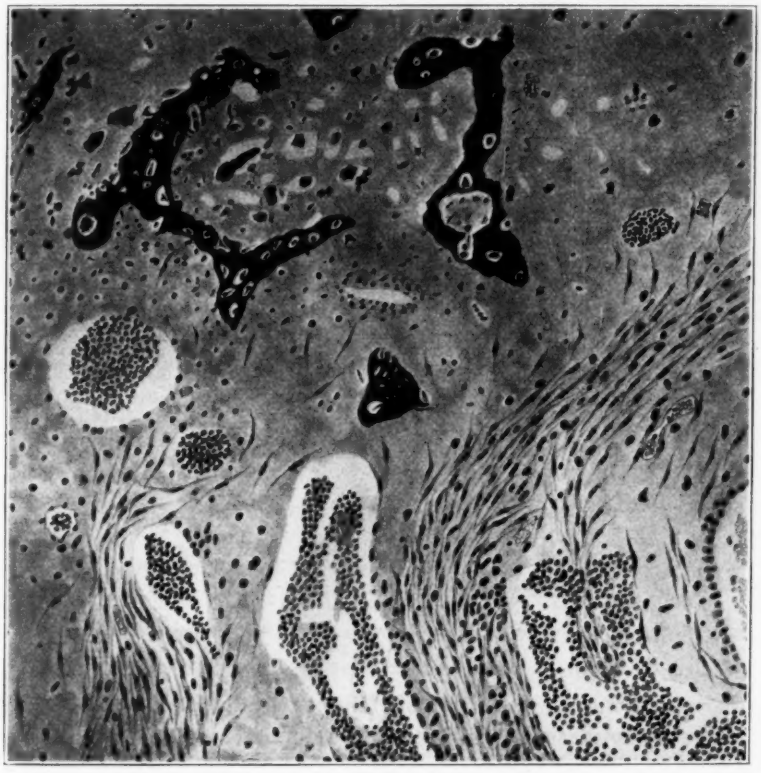

Fig. 2.—Illustrating the bone, smooth muscle, and endometrium in the mixed tumor of the uterus.

constricting its lumen. This tumor microscopically contains large masses of fatty areolar tissue separated by bands of fibrous tissue in which there are small groups of cartilage cells and narrow bands of smooth muscle cells. (Fig. 3.) About 1 per cent of the tissue is cartilage and smooth muscle fibers, 5 per cent fibrous tissue, and 94 per cent fatty areolar tissue. The patient is living and well two years after the operation and six years after the appearance of symptoms.

Wilms' explanation of the origin of mixed tumors of the uterus by displacement of embryonal rests along the Wolffian duct is generally accepted. In the female, the Wolffian duct disappears, or only vestigial remnants per-

sist as Gärtner's duct.* When complete it passes from the epoöphoron through the broad ligament, at first parallel with the fallopian tube, then more diagonally as the base of a triangle whose opposite angle is formed by the uterine portion of the fallopian tube and the body of the uterus.

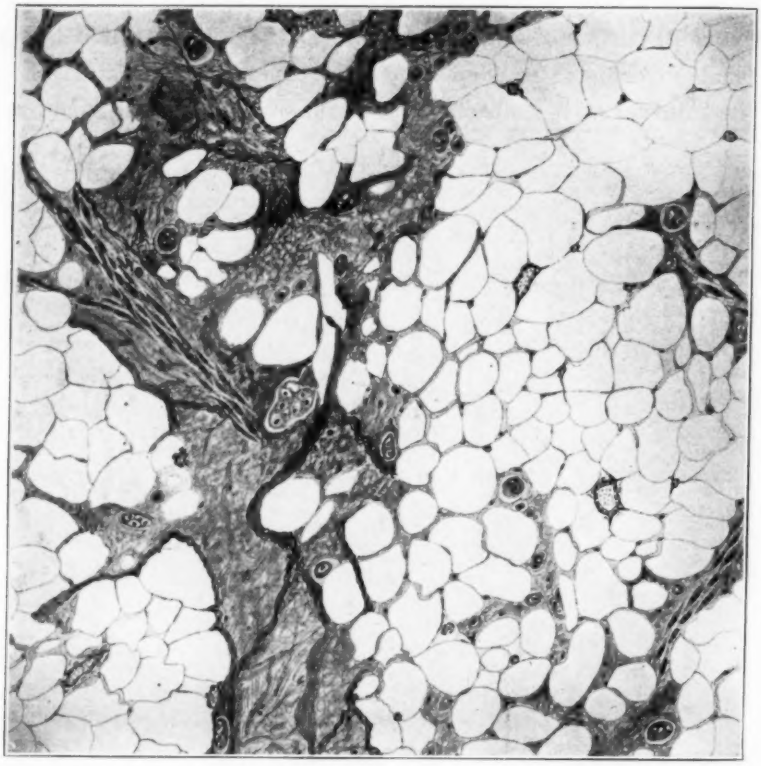

Fig. 3.—Representing the large areas of fat with bands of smooth muscle and fibrous tissue, and areas of cartilage.

Near the level of the internal os the duct passes into the uterine substance, gradually approaching but not reaching the mucosa. Beyond the uterus it may extend in the lateral wall of the vagina to the hymen.

*Kiebel and Mall: Handbuch der Entwickelungsgeschichte des Menschen. Leipz., ii, 1911.

THE PSEUDOSPIROCHETES DERIVED FROM RED BLOOD CELLS*

By Edwin W. Schultz, M.D., Stanford University, California

INVESTIGATORS have not infrequently been misled by pseudoparasites. A large variety of artifacts, both of endogenous and exogenous origin, have been described as parasites of one sort or another. A pseudoparasite of particular interest, because of its apparently animated behavior, is the spirochete-like body which under certain conditions is extruded from the red blood cell. Its morphology and motility are such that the uninitiated will probably always consider it some new parasite. Indeed, as I shall point out later, several investigators have already given it a place among the parasites and also assigned to it a rôle in disease. It is not unlikely that more such mistakes would be made were the dark-field more generally employed in the examination of blood, or blood fluids. Since much of the literature bearing on this remarkable phenomenon is obscured by misleading titles, a review of the papers which have come to my notice may prove both interesting and instructive. A more general knowledge of the phenomenon among clinical laboratory workers and investigators may serve to forestall further misinterpretation of these spirochete-like bodies.

In 1863, Beale[1] read a paper before the Royal Microscopical Society of London in which he described for the first time this peculiar type of cellular disintegration. The phenomenon in question manifested itself following "the application of a gentle heat" to the preparations. He says: "Under these circumstances long and very fine threads are, as it were, drawn out from the red viscid matter, and these threads exhibited perpetual vibratory movements I have seen them oscillating in many different places at the same time, like minute vegetable threads developed in the mouth (Leptomitus?). Not only so, but many exhibit a distinctly headed appearance; and when these filaments are detached, they certainly very closely resemble bacteria." These changes were observed in both human and frog blood. The following year Preyer[2] described similar observations on extravasated blood of the frog and salamander. He also observed that the phenomenon is promptly induced when a drop of blood is placed on a slide coated with a thin layer of crystallized urea. Schultze[3] in 1865 made a careful study of the influence of temperature on red blood cells, employing a specially designed thermo-objective which was lowered into a small moist chamber containing the preparation; essentially a warm stage of the present day. By this means he was able to observe accurately the effects of given increases of temperature on the cells. His studies were limited to human blood. The most pronounced effects were noted when the temperature reached 52° C. Marked

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alterations in the shape of the red cells occurred. In some cases the corpuscles became drawn out into long cylinders, with terminal enlargements; other corpuscles were transformed into long threads with bead-like varicosities, and still others developed one or more long filaments. These filaments showed lively serpentine motion, even after they became detached from the corpuscle. Schultze also observed that the red cells in the blood from patients with high fevers were much more prone to undergo deformities than those in blood from normal individuals. This was also observed subsequently by other investigators. Arndt[4] in particular noted the greater facility with which the red cells from febrile cases gave rise to filamentous extrusions, and other types of disintegration. He was especially impressed by the marked deformities of the red cells from a case of relapsing fever, which he observed at the time of a paroxysm. Because of his observations he doubted the parasitic nature of the spirochetes just previously described in relapsing fever, considering it more likely that they were produced by the red cells under the influence of fever and consequent nutritional disturbances. Such views were also expressed by Guttmann[5] the following year. Albrecht,[6] on the other hand, with the aid of these disintegration products pieced together a well thought out life cycle for S. obermeieri.

The phenomenon has also been described a number of times in more recent years, and in practically every case as observations which were new to the investigator. One of the most interesting of these is by Nuttall and Graham-Smith[7] in 1907. Their observations were made while studying canine piroplasmosis. It is worthy of note that the phenomenon was the cause of much trouble to these experienced investigators, until they "succeeded in demonstrating that these bodies are not parasites, but degeneration products of red blood corpuscles." The cause was traced to retained heat in the platinum loop used in transferring a drop of blood to a slide. The effect of heat on red blood cells was thereupon carefully studied. They observed that in human blood the most marked changes occurred when the temperature was raised to between 51.5° and 52.5° C. The corpuscles of guinea pigs, rabbits and dogs withstood a slightly higher temperature (54° C.). Later in the same year, Eve[8] described the origin of these spirochete-like bodies from the red and white cells in several pathologic cerebrospinal fluids. The first instance was in the cerebrospinal fluid of a chimpanzee affected with cerebral syphilis. "Attached to some of the red corpuscles were minute filaments showing active streaming movements, and resembling in some ways the living Spirocheta pallida." Motile filaments were also observed in the spinal fluid of one out of ten general paralytics he examined. Most of the remainder showed other degeneration products—globular bodies united by short filaments, etc. He observed that white as well as red cells may flagellate. This has also been pointed out by other investigators (see Balfour[10]), but flagellation of the red cells seems to be far more common. Porter,[9] also in 1907, observed the phenomenon in blood obtained by finger puncture, which he sealed between a slide and cover-slip and left for a number of hours at room temperature. In addition to corpuscles "with fine hair-like processes,"

he observed bodies which were "not unlike a tadpole in appearance, and moved by a lashing of the tail," and likewise other odd forms, much like those described by earlier workers. Balfour[10] in 1911 described the phenomenon in an excellent article on blood artifacts, which every novice should read. He refers to the various types of corpuscular disintegrations as the "chain, dumb-bell, droplet, and filament phenomenon," a term which is indeed descriptive. Balfour points out that while the influence of heat on the cells is an important factor in the production of these filaments, "it is evident that they may be seen by the dark-field method when there is no possibility of the blood temperature having been raised, at least to any appreciable extent." Balfour's references to the observations of Crawley, Ross and Macalister, and Buchanan, I have been unable to locate in the literature. Seidlin[11] the following year in a report on his yellow-fever studies states that he observed by the dark-field method "some peculiar filaments" in the blood of several cases of yellow-fever, and later in the blood of normal individuals. "They appeared at first as small, extracorpuscular rods, which in some cases seemed to take their origin from small, free rings; they developed subsequently into fine filaments, the length of which varied from one-fourth to almost the entire diameter of an erythrocyte. They had generally, a distinct dot in one or, more frequently, in both extremities, and were very motile, possessing undulating, distinctly progressive movements." He did, however, not recognize the ultimate source of these bodies, as the following lines reveal: "The nature of these filaments remains entirely obscure. They have certainly nothing to do with the red blood corpuscles, and are thus different from the bodies described by Nuttall and Graham-Smith (1906), and Balfour (1911). The only relation to blood elements which I can admit as possible, is that they might take their origin from platelets; the ring-shaped bodies, from which they were seen to develop were not wholly unlike these elements. This origin, however, would not account for their peculiar shape and characteristic movements, features which surely suggest living organisms." What he undoubtedly observed were the flagellated droplets extruded from the red blood corpuscles. These are common in blood subjected to momentary heat. That they "suggest living organisms" is certainly true. Gastou,[12] in his little book on the dark-field, published in 1912, illustrates the common morphologic types of these filaments. In 1920, Eberson,[13] without reference to any of the earlier work, again described the phenomenon. He produced these filamentous extrusions from the red corpuscles by altering their physicochemical environment; that is, by changing the tonicity or hydrogen-ion concentration. He found, in fact, that simply transferring the cells from their normal environment was all that was necessary to induce the phenomenon. He discovered, however, that it was distinctly accelerated by certain tissue extracts. A little testicular extract caused an abrupt transformation of the cells. He mentions that "extremely tenuous, filamentous forms" are occasionally seen in testicular fluid obtained by puncture; "findings that have led some workers to suggest

these appearances as phases of the life-cycle of the organism causing syphilis." This again illustrates the practical bearing of the phenomenon.

To illustrate how easily the unwary investigator may be misled by these bodies, let me cite some misinterpretations which have been made. In 1892, Thoinot and Calmette[14] announced that they had discovered actively motile, flexile, threads in the blood of typhus fever patients and cadavers. These bodies measured from ten to thirty microns in length and moved in a rapid serpentine fashion among the corpuscles. These filaments often showed more or less prominent terminal enlargements. The longer filaments, in addition to the terminal enlargements, showed secondary enlargements scattered along intermediate points. These, they state, were of the same refractile character as the terminal enlargements, but of a smaller size. These I believe were undoubtedly the varicose forms frequently present among degenerating red blood cells. Similar filaments were seen attached to red blood cells, but these showed no active motion. What they undoubtedly observed here was the production of the filaments. Unfortunately their illustrations were sketched from memory some time after the observations were made, and do not depict the filaments in their true form. Their description, however, leaves no doubt in my mind as to what they actually saw. It is significant that though they were fully cognizant of the aforementioned observations by Schultze, Preyer, and Arndt, they were nevertheless inclined to regard these bodies as "éléments spécifiques." Two months later a paper by Lewaschew[15] appeared showing that this investigator had fallen into the same error. Lewaschew even went so far as to propose the name "Spirochete exanthematicum" for these filaments. He speaks of cocci, cocci with threads, and free threads, considering it probable that these are stages of the same organism. Some of the threads showed varicosities; others a single terminal enlargement, while the free threads were of uniform contour. It is interesting that he observed these bodies in increasing numbers as the disease progressed, and that the number and their activity rapidly decreased as the temperature dropped, and after the crisis almost entirely disappeared. I have already referred to the apparent relationship between fevers and this phenomenon. In 1919 I examined about fifty specimens of blood from cases of typhus fever with the darkfield. These pseudospirochetes were present in large numbers in practically every specimen examined.

In 1912, Dr. Chambers[16] described them as a new spirochete of the human blood stream. These she observed in the blood from cases of Graves' disease, and in the blood from healthy individuals. She described them as actively motile; quite variable in both length and thickness; body soft and flexible, forming open curves, which were constantly obliterated and reformed, and possessing terminal enlargements. She believed that the thinner forms were difficult to distinguish from Treponema pallidum. Some were seen to be attached to red blood cells. She believed that she had succeeded in cultivating them. The "spirocheta," however, multiplied only in the original blood clot. This to my mind means that as the corpuscles degenerated, pseudospirochetes were formed. I have observed them in large numbers

in the blood from typhus fever patients which had been left in an incubator overnight or longer. Dr. Chambers stained the "spirocheta" by the Leishman, Giemsa, and Levaditi's pyridin methods. Porter[17] shortly after pointed out the true nature of the supposedly new organism Dr. Chambers described. He says: "The bodies Dr. Chambers described I observed originally in the urine and I thought I had discovered a new organism till I found them in the blood and discovered what I believe to be their true significance. In certain anemias where poikilocytosis is well marked, the pointed end is an extrusion from the corpuscle and has the power of movement. This elongates and is finally detached into one of Dr. Chambers' thick form of 'spirocheta' and by careful observation this will be found in time to elongate into finer 'spirocheta.'"

In 1916 Korbsch[18] in the German Army described what he presumed was

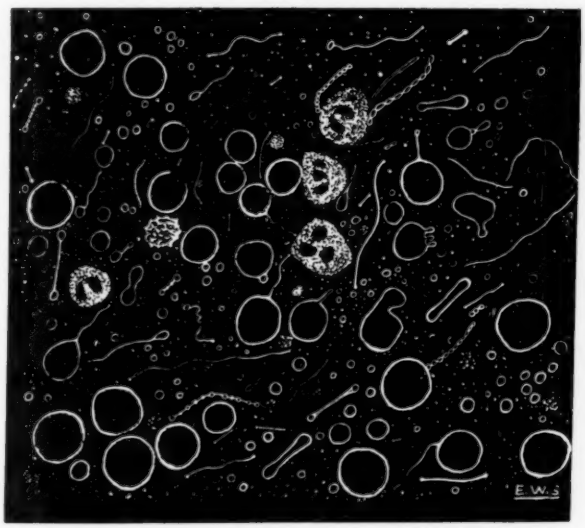

Fig. 1.—Pseudospirochetes and other products of corpuscular disintegration following the application of a hot platinum loop to a drop of blood.

a spirochete in the blood of a then new disease, resembling relapsing fever, later designated Wolhynian fever, trench fever, etc. Because of the efficacy of arsenic therapy in the disease he sought for a spirillum as the most likely cause. His search for spirillum was rewarded. He observed actively lashing filaments, measuring about ten microns in length, among the erythrocytes. In the Giemsa preparations these stained a pale grayish-blue, occasionally a distinct reddish violet, and showed for the most part reddish granules. There were both stretched and coiled filaments. The bodies were found on the febrile days, especially towards evening when fevers are generally highest. We see again the relationship of the phenomenon to fevers, though I have no doubt that diligent search with the dark-field will reveal them at any time. That the phenomenon is prominent also in trench fever is indicated by a remark made by Jungmann and Kuczynski[19] in a report of their studies

on the etiology of the disease, to the effect that they observed the production of the pseudospirochetes in abundance.

Although one unfamiliar with these pseudospirochetes might take them for new parasites, there should be no difficulty in differentiating them from Treponema pallidum. Shortly after Eberson's paper appeared, Sutton,[20] as a safeguard, advised the use of distilled water, instead of physiologic salt solution, which I[21] had recommended, as a vehicle in aspirating the pulp from regional lymph glands for dark-field examination in suspected primary syphilis. However, laking the blood does not entirely prevent the production of the pseudospirochetes. I have observed them in blood laked purposely to determine this point. Therefore, the real safeguard rests on an acquaintance with these bodies. Fortunately they are rarely seen in the routine dark-field examination for primary syphilis. In the course of more than fifteen hundred dark-field examinations for primary syphilis made while in the army, I observed these spirochete-like bodies only twice. I never observed them in the aspirated pulp from regional lymph glands. The reason for the rarity of pseudospirochetes in the usual dark-field examination for syphilis undoubtedly rests on the brevity of the procedure. Though red cells may flagellate immediately after they are subjected to deleterious influences, under more tolerable conditions this takes place slowly.

These pseudospirochetes vary considerably in morphology. They vary in length from less than five microns to more than forty microns. Usually they measure between ten and twenty microns in length. No relationship exists between length and thickness. Some are very long and thin, while others are short and thick. Between these two extremes, may be found all possible combinations of length and thickness. The majority measure about 0.5 micron in thickness, and when viewed with the dark-field, the entire thickness of the body is illuminated. The exceptionally thick ones show only marginal illumination, as do red blood cells, droplets, and the larger bacteria. The filaments usually have more or less marked terminal enlargements; this is in the form of either a dot, bulb, or globule. In some these are present at both ends, in others at one end only, and rarely the filaments terminate bluntly without any enlargement whatever. The filaments, however, never taper to a point. The terminal dots and knobs are slightly more refractile than the rest of the filament; the globular ones, however, show only marginal illumination. The filaments are otherwise of uniform thickness and even contour. They are never cork-screw spiral in shape. The filaments eventually develop varicosities and break up into coccoid bodies. These varicose forms often resemble chains of streptococci. Besides the free filaments, one may observe various bizarre bodies, globoid bodies with flagellae, chains, dumb-bells, etc.

The movements of these pseudospirochetes give one the impression of living organisms. These vary somewhat, depending upon the dimensions of the filaments. The long delicate threads exhibit a streaming motion, with gentle open curves. Those somewhat thicker exhibit serpentine, or lashing movements. Others, apparently more rigid, make only slight bending move-

ments; while still others lie relatively quiet. The most common movements are serpentine and moderately progressive. These movements are, of course, dependent upon physical laws and not upon living forces.

The differences between these pseudospirochetes and Treponema pallidum are briefly as follows: (1) The pseudospirochetes are not cork-screw spiral in shape. (2) They do not have tapering ends. (3) Their bodies are flexile and when in motion form open curves. (4) They never exhibit rotary motion around their long axes, with forward and then backward movements.

A rapid way to demonstrate the phenomenon is to place a small drop of blood on a slide; touch the drop with a hot platinum loop, drop on a cover-slip and examine with the dark-field. To prepare stained preparations, place the drop at one end of the slide; touch with hot platinum loop; smear with the edge of another slide, and stain according to either Jenner's, Wright's, or Giemsa's method. The filaments take the same color as the red blood corpuscles, and correspond in morphology to those observed in the dark-field.

BIBLIOGRAPHY

[1]Beale, L. S.: Observations Upon the Nature of the Red Blood Corpuscle; Trans. Microscop. Soc., Lond., 1864, xii, N.S., 37.

[2]Preyer, W.: Ueber amöboide Blutkörperchen, Virchow's Arch. path. Anat., Berlin, 1864; xxx, 417.

[3]Schultze, M.: Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes, Arch. f. mikroskop, Anat., Bonn, 1865, i, 1.

[4]Arndt, R.: Beobachtungen an rothen Blutkörperchen der Wirbelthiere; Virchow's Arch. f. path. Anat., Berlin, 1879, lxxviii, 1.

[5]Guttmann, P.: Zur Histologie des Blutes bei Febris recurrens, Ibid, 1880, lxxx, 1.

[6]Albrecht, R.: Beitrage zur Kenntnis und Entwicklung der Spirochaete obermeieri, Deutsch. Arch. f. klin. Med., Leipsic, 1881, xxix, 77.

[7]Nuttall, G. H. F. and Graham-Smith, G. S.: Canine Piroplasmosis, Jour. Hygiene, Cambridge, 1907, vii, 268.

[8]Eve, F. C.: Some Motile Elements seen in Certain Cerebrospinal Fluids; Brit. Med. Jour., Lond., 1907, ii, 1399.

[9]Porter, F.: Observations on Fresh Blood Films with Special Reference to Hemoconia, Ibid, p. 1773.

[10]Balfour, A.: Fallacies and Puzzles in Blood Examination; Fourth Report, Wellcome Trop. Res. Laboratories, Lond., 1911, Vol. A.—Medical, p. 115.

[11]Seidelin, H.: Report of Yellow-Fever Expedition to Yucatan, 1911-12; Yellow Fever Bur. Bull., Livrpl., 1912, ii, 187.

[12]Gastou, P.: L'Ultra-Microscope, Ed. 2, Paris, 1912.

[13]Eberson, F.: "Spirochetes" Derived from Red Blood Corpuscles; Arch. Dermat. and Syph., Chicago, 1920; l, N.S., 638.

[14]Thoinot, L. H. and Calmette, E.: Note sur quelques examens de sang dans le typhus exanthematique, Ann. de l' Inst. Pasteur, 1892, vi, 39.

[15]Lewaschew, S.: Ueber die Mikroorganismen des Flecktyphus, Deutsch. med. Wchnschr., 1892, xviii, 279.

[16]Chambers, H.: A New Spirochaeta Found in the Human Blood; Lancet, London, 1913; i, 1728.

[17]Porter, F.: A New Spirochaeta Found in the Human Blood, Ibid, ii, 102.

[18]Korbsch, R.: Ueber eine neue dem Rückfallfieber ähnliche Kriegskrankheit, Deutsch. med. Wchnschr., 1916, xlii, 343.

[19]Jungmann, P. and Kuczynski, M. H.: Zur Klinik und Aetiologie der Febris wolhynica (His-Wernersche Krankheit), Ibid, vliii, 359.

[20]Sutton, I. C.: A New Method for Demonstrating Spirochetes by Lymph Gland Puncture, Jour. Am. Med. Assn., 1921, lxxvii, 1889.

[21]Schultz., E. W.: A Method for Demonstrating Treponema pallidum in Regional Lymph Glands, Jour. Am. Med. Assn., 1920, lxxv, 176.

THE PHARMACOLOGY OF ISOPROPYL ALCOHOL*

A Synopsis of Available Data

By Dudley H. Grant, Elizabeth, N. J.

ISOPROPYL, or secondary propyl, alcohol has recently come into commerce, both in America and abroad. Recent articles in technical journals indicate that it is coming into use in Europe as an ingredient of various preparations for cosmetic and external medicinal uses, and give reason to suppose that it will find such use in this country[1]. The question of the physiologic effects of this alcohol, when ingested or applied to the skin or mucous membranes, is therefore timely and of some importance. A thorough search of the available literature, together with the results of some unpublished investigations, is epitomized below.

Isopropyl alcohol is a homolog of ethyl alcohol, having the empirical formula C_3H_7OH, and isomeric with normal propyl alcohol. It is produced in this country as a by-product of the petroleum or natural gas industry and in Germany by the reduction of acetone.

Isopropyl alcohol is similar in most of its properties to ethyl alcohol. The commercial alcohol, which is a constant-boiling mixture containing 91 per cent alcohol and 9 per cent water, boils at 80.4° C. and has a specific gravity of 0.816 at 20° C. The odor of isopropyl alcohol is different from that of ethyl alcohol, lacking the vinous quality, while its taste is distinctly disagreeable except in high dilution.

EARLY INVESTIGATIONS

The value of many of the earlier data on the toxicity of this substance is impaired by the doubtful purity of the alcohol employed.

Dujardin-Beaumetz and Audigé[2], by subcutaneous injection in dogs, found both isopropyl and normal propyl alcohols to be about twice as toxic as ethyl alcohol.

Ringer and Sainsbury[3] investigated the depressant effect of various alcohols on the isolated hearts of frogs, and found isopropyl alcohol to be about two and one-half times as toxic as ethyl alcohol.

Efron[4] recorded the reaction to galvanic stimulation of frog's nerve-muscle preparations immersed in the dilute alcohols. He found the toxicity of isopropyl alcohol to lie between that of ethyl and that of normal propyl alcohol.

Schneegans and von Mering[5] administered 4 grams of isopropyl alcohol to a rabbit and observed narcotic symptoms lasting 2 hours, followed by complete recovery.

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Vandevelde[6] compared various alcohols by their effects on plasmolysis in onion cells and on hemolysis in human and bovine blood corpuscles. He found isopropyl alcohol to be from one-third to one-half more toxic than ethyl alcohol, and about two-thirds as toxic as normal propyl alcohol, when used alone. In solution in ethyl alcohol, however, the toxicity of all the higher alcohols was increased.

Kemp,[7] by galvanic stimulation of frog's muscles, showed isopropyl alcohol to be less toxic than normal propyl alcohol.

RECENT AMERICAN WORK

Since isopropyl alcohol has come into production on a commercial scale, its pharmacologic properties have been more rigorously investigated.

The work[8] of Dr. David I. Macht of Johns Hopkins University may be summarized as follows:

Comparison of the lethal doses indicates that isopropyl alcohol is twice as toxic as ethyl alcohol when administered intravenously to cats. The comparative depressant action of the alcohols on the isolated frog's heart and on isolated plain muscle (pig's ureter) showed a similar ratio of toxicities.

Rats exposed to air saturated with methyl alcohol vapor died in a day or two, while both ethyl and isopropyl alcohol left them uninjured after several days*, causing, moreover, no blindness nor defect in vision.

Subsequent work by Dr. Macht[9] shows the minimum lethal dose of orally administered isopropyl alcohol, for cats, to be about 6 c.c. per kilo, while the same dose administered to dogs caused only a narcotic effect. Repeated sponging of the shaved skin of rats, dogs and rabbits with the alcohol caused no toxic symptoms, either local or systemic, nor any interference with the growth of hair. Of a number of rats having the posterior half of the body dipped repeatedly into isopropyl alcohol, practically all survived, notwithstanding possible absorption through the anus.

The narcotic effect of inhaled and of intraperitoneally injected isopropyl alcohol was tested by the behavior of trained white rats in the circular maze. The narcotic dose was found to be exactly half that of ethyl alcohol.

Dr. R. Burton-Opitz, of Columbia University, has also investigated† the toxicity of isopropyl alcohol.

Isopropyl and ethyl alcohols of various concentrations were injected into the jugular vein of dogs, and the carotid arterial pressure recorded. The depressant action of the two alcohols was found to be approximately equal.

Isopropyl and ethyl alcohols were found to have about the same effect when instilled, in various concentrations, into the conjunctival sac.

Rabbits and dogs inhaling air saturated with isopropyl alcohol vapor for an hour showed only a transient unsteadiness of gait.

Administration of isopropyl alcohol by stomach tube to a dog in a dose of 7.7 c.c. per kilo body weight produced severe narcotic symptoms, with complete recovery.

*Personally communicated.

†Unpublished in extenso, but abstracted in American Perfumer, 1921, xvi, 334.

Dilute isopropyl alcohol was found to possess healing properties similar to those of ethyl alcohol when applied to wounds on dogs.

Dr. Burton-Opitz has performed further work on this subject, and will publish it in the near future.

Dr. W. Gilman Thompson* administered isopropyl alcohol to human subjects. Each of six volunteers drank 25 c.c. of isopropyl alcohol (22.8 c.c. on an absolute basis), suitably diluted. One-half hour after the ingestion of the alcohol a diminution of the systolic blood pressure varying from 6 to 20 mm. was noted. In four cases, there was an increase in the pulse rate of from 6 to 14 beats per minute. Three of the subjects noted a slight dizziness. In no case was there any mental exhilaration such as would be produced by taking ethyl alcohol, nor was there any mental disturbance or muscular incoordination. After two or three hours, all of the men complained of headache, mild and transient in two cases, more severe in two cases, and very severe in the last two so that the subjects did not recover for about 24 hours. Practically all the men felt mentally depressed.

The effect of isopropyl alcohol on the human skin was tested under the supervision of Dr. J. M. Sinclair,† of this Company. Five volunteers wore bandages saturated with undiluted isopropyl alcohol on one forearm, covering an average area of 60 square inches, for four daily periods, aggregating 21 hours for each man. No harmful effects, either local or systemic, were noted, other than a slight maceration of the outermost layer of the epidermis, unaccompanied by irritation.

Isopropyl alcohol has been employed by members of this laboratory, during the past three years, in lotions, liniments, liquid soap, mouth washes and gargles. It has been used undiluted to cleanse small wounds and to dry and harden the skin of hands swollen by hot water or alkali. The present writer has frequently taken sponge baths, from head to foot, in isopropyl alcohol of about 30-50 per cent concentration, which was found to be remarkably cleansing. In no case has any injurious effect been noted. The alcohol has been recently used by several nurses and hospitals for alcohol rubs on a number of patients, under medical supervision. No untoward effects have been reported.

It should be noted that all the recent American work described above has been done with the commercial 91 per cent isopropyl alcohol. All the dilutions mentioned are on this basis, and do not refer to absolute alcohol.

GERMAN INVESTIGATIONS

The following recent papers from German medical and technical journals are of interest.

Dr. H. Boruttau of Berlin University[10] bound the shaved abdomens of rabbits and the forearms of human subjects with bandages saturated with 60 per cent isopropyl alcohol. These were worn several hours daily for 14 days. Aside from a slight erythema and superficial maceration, exactly similar in degree to that caused by ethyl alcohol in control experiments, no pathologic symptom or other disturbance of normal health was noted.

*Personally communicated.
†Personally communicated.

The minimum lethal dose of isopropyl alcohol, by stomach tube to rabbits, was 5 grams per kilo, the same as that of ethyl alcohol.

Boruttau drank 5 c.c. of isopropyl alcohol himself three times, without injury. He also employed it in hand-disinfection and in the preparation of a mouth-wash, without ill effect. He concludes that "there is no reason why isopropyl alcohol should not be employed for cosmetic and hygienic purposes, so long as it comes in contact with the epidermis, or temporarily with the mucous membranes, and no harm need be feared from the accidental swallowing of moderate quantities of this alcohol."

Dr. K. Loeffl[11], together with two companions, drank a liqueur containing 4 c.c. each of isopropyl and ethyl alcohols. On successive days the dose was doubled, and finally 40 c.c. of a liqueur containing 40 per cent isopropyl alcohol was taken daily for three days. This corresponds to 16 c.c. of pure isopropyl alcohol. No harmful effect was observed. Loeffl says: "I am convinced that no harmful effect can result from the use of isopropyl alcohol for external washes or from the small quantities which may be absorbed through the skin or in the stomach during the use of mouth washes, dental solutions or hair preparations. The use of isopropyl alcohol for these purposes is therefore justified."

Dr. J. Pohl[12] of Breslau University investigated the metabolic transformation of isopropyl alcohol in rabbits and dogs, by analysis of the exhaled air. About 12 to 13 per cent of the original dose was exhaled as acetone and unchanged isopropyl alcohol. Analysis of the liver of one dog killed after twelve hours indicated only a slight retention of the alcohol. Pohl considered that a negative copper-reduction test on the urine indicated the absence of conjugated glycuronic acids. This is in contradiction to the work of Neubauer[13], who found part of the isopropyl alcohol ingested by rabbits and dogs to be excreted in combination with glycuronic acid.

Pohl states: "I consider myself justified in concluding, subject to the reservation that the exhalation should perhaps have continued longer than 12 hours, that small doses of isopropyl alcohol, incapable of producing narcotic symptoms, are transformed into heat in the body, that is, oxidized, to the extent of over 80 per cent. Isopropyl alcohol therefore approximates ethyl alcohol, of which, as is well known, over 90 per cent is burnt up in the metabolism".

Pohl also performed an experiment on the effect of repeated small doses of isopropyl alcohol on growth. Two puppies from the same litter were given repeated small doses, totalling 224 c.c., of isopropyl alcohol and water, respectively. The alcoholized puppy gained 220 grams more than the other, during two months.

A large dog in nitrogen equilibrium was given 20 c.c. of isopropyl alcohol, and the total nitrogen in the urine determined for a two-day period before and after ingestion of the alcohol. Only an insignificant increase was observed.

The antiseptic and bactericidal action of isopropyl alcohol has been investigated by Bernhardt[14] and others, whose work will shortly be reported by the present writer[15].

SUMMARY

The toxicity of isopropyl alcohol is not less than that of ethyl alcohol and not more than twice that of ethyl alcohol. These limits include all the reliable data at hand, by six different observers, using a total of nine different methods, on one or another of six different animals.

The effect of isopropyl alcohol upon the human skin is similar to that of ethyl alcohol.

Different observers do not agree upon the effect of large doses of isopropyl alcohol orally ingested by human subjects. Loeffl's investigators took 16 c.c. daily for three days without discomfort, while Thompson's subjects suffered marked, though not dangerous, depression from a single dose of 22.8 c.c. All are agreed, however, upon the absence of any exhilarating effect similar to that of ethyl alcohol, and upon the somewhat unpleasant taste of isopropyl alcohol. It may, therefore, be safely said that isopropyl alcohol is not potable.

In view of all the foregoing data, no reasonable objection can be made to the use of isopropyl alcohol as a lotion or vehicle for external medication, nor as a constituent of compounds for oral and nasal medication, such as practically all mouth washes, gargles, dentifrices, etc., which do not involve the probability of swallowing more than a few cubic centimeters, at most, of the alcohol.

BIBLIOGRAPHY

[1]Grant, D. H., and Johns, C. O.: Isopropyl Alcohol, Am. Jour. of Pharmacy, 1922, xciv, 418.
[2]Dujardin-Beaumetz and Audigé: Recherches Expérimentales sur la Puissance Toxique des Alcools. Paris, Octave Doin, 1879.
[3]Ringer, S., and Sainsbury, H.: Observations on the Relative Effects of Certain Members of the Ethylic Alcohol Series on the Ventricle of the Frog's Heart, Practitioner, London, 1883, xxx, 339.
[4]Efron, J.: Beitraege zur allgemeinen Nervenphysiologie, Archiv fuer Physiologie, ed. Pflueger, 1885, xxxvi, 467.
[5]Schneegans, A., and von Mering, J.: Ueber die Beziehungen zwischen chemischer Constitution und hypnotischer Wirkung, Therapeutische Monatshefte, 1892, vi, 327.
[6]Vandevelde, A. J. J.: Détermination du pouvoir toxique des alcools monoatomiques par la plasmolyse. Archives Internationales de Pharmacodynamie et de Thérapie, 1900, vii, 123. Application de l'Hémolyse à la Détermination de la Toxicité des Alcools, Annales de la Société Médicale de Gand, 1905, lxxxv, 37.
[7]Kemp, H. P.: The Physiological Effects of Primary and Secondary Propyl Alcohol, etc., Proceedings of the Physiological Society, p. xlix, Jour. of Physiology, 1908, xxxvii.
[8]Macht, D. I.: A Toxicological Study of Some Alcohols, with Especial Reference to Isomers, Jour. Pharm. and Exper. Therapeutics, 1920, xvi, 1.
[9]Macht, D. I.: Pharmacological Examination of Isopropyl Alcohol, Arch. Internat. de Pharm. et de Thérapie, 1921, xxvi, 285.
[10]Boruttau, H.: Die Verwendung von Isopropylalkohol zu hygienischen und kosmetischen Zwecken, Deutsch. Med. Wchnschr., 1921, xlvii, 747. Translated in American Perfumer, 1922, xvi, 523.
[11]Loeffl, K.: Isopropylalkohol, Seifensiederzeitung, 1921, xlviii, 542.
[12]Pohl, J.: Zur Kenntnis des Methyl- und Isopropylalkoholschicksals, Biochemische Zeitschrift, 1922, cxxvii, 66. Quantitative Versuche ueber die Exhalation von Alkoholen, Archiv fuer experimen. Path. u. Pharm., Supplement-band 1908, 427.
[13]Neubauer, O.: Ueber Glykuronsaeurepaarung bei Stoffen der Fettreihe, Archiv. f. experimen. Path. u. Pharmakol., 1901, xlvi, 133.
[14]Bernhardt, G.: Ueber Isopropylalkohol als Mittel zur Haendedisinfektion, Deutsche Med. Wchnschr., 1922, xlviii, 68.
[15]Grant, D. H.: The Antiseptic and Bactericidal Properties of Isopropyl Alcohol, The American Journal of The Medical Sciences (publication pending).

THE PATHOGENICITY OF STREPTOCOCCI FROM DIPHTHERIA THROATS FOR MICE*

By Louis Brody and Lloyd Arnold, M.D., Chicago, Ill.

SOME interesting changes in the streptococci flora of the upper respiratory tract during attacks of diphtheria are recorded in another paper in this number.[1] One of the most striking differences observed was the change in the limiting hydrogen-ion concentration of the strains isolated from the throats of diphtheria patients. The work reported in this paper was undertaken for the purpose of testing the pathogenicity for white mice of strains of streptococci isolated from the diphtheria throats.

Historical.—Smillie,[7] using Henderson and Palmer's colorimetric method for the hydrogen-ion determination, found 4 strains of hemolytic streptococci isolated from the throats of scarlet fever patients to have a limiting hydrogen-ion concentration of P_H 5.1 to 5.7, all 4 strains were pathogenic for mice; one strain having a limiting hydrogen-ion concentration of P_H 4.5 was not pathogenic for a mouse. Jones[5] isolated 13 strains of hemolytic streptococci from milk, producing a limiting hydrogen-ion concentration of P_H 5.0 to 5.2; five of these were tested as to their pathogenicity for rabbits; the results were negative.

Technic.—In the winter of 1921-22 we obtained, through the courtesy of the City Board of Health, Chicago, some fifty Loefflers-serum cultures taken from throats of cases showing a positive B. diphtheria growth. These cultures were obtained a few hours after they had been received at the City Board of Health Laboratory. A platinum loop was used to transfer some of the growth from the Loefflers-serum slants to blood agar plates, otherwise the technic was the same as described in the previous papers.[1] The limiting hydrogen-ion determination was made by the colorimetric method. While the work with these strains of streptococci was in progress, we had, in connection with another problem, the opportunity of using the colorimetric methods of Clark and Lubs,[3] Barnett and Chapmann,[2] Medallia[6] and Gillispie.[4] If the same quality of glassware was used and the same distilled water for all the dilutions, the four methods checked within P_H 0.2 of each other within the limits used for this work. For most of this work the Clark and Lubs method was used.

Experiments.—Mice were used to test the pathogenicity of the strains of streptococci. Two mice were used for each strain, one receiving 0.5 c.c., the other 1 c.c. of a 24-hour growth of 1 per cent dextrose hormone bouillon, injected subcutaneously in the back near the tail.

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Twenty strains of hemolytic streptococci from diphtheria throats were used, the limiting hydrogen-ion concentration varying from P_H 4.2 to 5.6. One strain with a limiting H-ion of P_H 4.6 killed the mice with both 0.5 and 1 c.c. injections within 24 hours, one strain with a limiting H-ion concentration of P_H 4.7 with 1 c.c. injection died four days later; the mouse receiving 0.5 c.c. of this strain was not affected. All the other 36 mice were living 6 weeks after the injection. Twenty strains of viridans streptococci from diphtheria throats were injected in the same manner, the limiting H-ion concentration varying from P_H 4.3 to 5.0. All 40 mice were alive 6 weeks later.

Twelve strains of hemolytic streptococci isolated from normal throats with a limiting H-ion concentration of P_H 4.9 to 5.2, and 20 viridans strains from the same source with a limiting H-ion concentration of P_H 4.3 to 5.0, were injected into mice in the same manner as the above diphtheria throat strains. All were negative as to their pathogenicity for mice.

SUMMARY

The streptococci isolated from the throats of diphtheria patients do not seem to be pathogenic for white mice. Twenty hemolytic strains, with the limiting hydrogen-ion concentration varying from P_H 4.3 to 5.6 were used, of these only two were virulent for the mice. These two strains had a limiting hydrogen-ion concentration of P_H 4.7 and 4.6 respectively, being midway between the pathogenic or human and the nonpathogenic or bovine types in this respect.

The 20 viridans strains, with a limiting hydrogen-ion concentration varying from P_H 4.2 to 5.0 were used; all were nonpathogenic for mice.

Hemolytic and viridans strains isolated from normal throats were used as controls. Hemolytic strains giving a limiting hydrogen-ion concentration within the human or pathogenic range were selected for this work All proved to be nonpathogenic for mice.

From the limited number of strains of streptococci, both hemolytic and nonhemolytic, isolated from throats of normal individuals and from diphtheria patients we were unable to find any relationship between the limiting hydrogen-ion concentration and their pathogenicity for white mice.

BIBLIOGRAPHY

[1]Arnold, L.: Jour. Lab. and Clin. Med., 192.
[2]Barnett, G. D. and Chapman, H. S.: Jour. Am. Med. Assn., 1918, lxx, 1062.
[3]Clark, Wm. and Lubs, H. A.: Jour. Bacteriol., 1917, ii, 1, 109, 191.
[4]Gillispie, L. J.: Spils, Soil. Science, 1920, ix, 115.
[5]Jones, F. S.: Jour. Exp. Med., 1920, xxxi, 347.
[6]Medallia, L. S.: Jour. of Bacteriol., 1920, v, 441.
[7]Smillie, W. G.: Jour. Inf. Dis., 1917, xx, 45.

CHANGES IN THE STREPTOCOCCI FLORA OF THE THROAT DURING DIPHTHERIA*

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WHILE studying the streptococci flora of the throats of children in a State Blind School, during the fall and winter of 1921, a few cases of diphtheria developed. There was a noticeable change in the streptococci of the throats of these cases. In this paper we wish to record the change we found during the period of observation.

Historical.—The increase in the number of streptococci found in smears from diphtheria patients' throats is a well-known observation. Loeffler[6] isolated and described streptococci from cases of diphtheria. The first to attach much importance to the streptococci of the diphtheria throat flora were Roux and Yersin, who attributed an increased virulence to the diphtheria bacilli when grown in symbiosis with streptococci. This question has since been studied by several investigators, and the literature has been collected by Bernheim,[3] Le Gros[5] and Ladendorff.[4]

Technic.—The technic was practically the same as that used in our former work.[1] The limiting hydrogen-ion concentration was determined according to the method of Avery and Cullen.[2]

Experiments.—There were twelve diphtheria and postdiphtheria throats studied; these did not differ markedly from the ones here recorded, but are omitted because the normal streptococci flora of the throat previous to the attack was not ascertained. The accompanying table only contains four of the cases studied, the others were practically the same type of streptococci flora.

Summary.—There is a marked increase in number of hemolytic streptococci in the throats of patients during diphtheria. The streptococci in the throat at this time have a limiting hydrogen-ion concentration differing from that of the normal throat flora, this is accompanied by an overgrowth of the types fermenting lactose and salicin. The strains that produce the limiting hydrogen-ion concentration between P_H 4.3 and 4.5 are considered by most investigators as of bovine origin, or nonpathogenic strains, and those reaching the lesser hydrogen-ion concentration limits, P_H 5.0 to 5.5 of human or pathogenic origin. If this is true the streptococci flora of the human throat can under normal conditions be considered as nonpathogenic types and the flora of the diphtheria throats as human or pathogenic types.

The limiting hydrogen-ion concentration must indicate in some way a result of a particular form of metabolic function of the streptococcus. Varia-

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tions in the environment of the organism, causing a change in its metabolism, should change to a certain extent its sensitiveness to a hydrogen-ion concentration.

	Case I					Case II				
	NO. STRAINS	LAC.	MAN.	SAL.	H-ION		LAC.	MAN.	SAL.	H-ION
5 days before exposure	1 B	+	–	+	4.3–4.5	0 B				
	3 Vir	+	–	+	4.3–4.5	2 Vir	+	–	+	4.3–4.5
	3 Vir	–	–	–	4.3–4.5	1 Vir	+	–	–	4.3–4.5
						1 Vir	–	–	–	4.3–4.5
Same day B. Diphtheria were culture from Throat	13 B	+	–	+	5.0–5.4	2 B	+	–	+	5.0–5.3
						1 B	+	–	+	4.3–4.5
						2 B	+	–	–	5.0–5.3
14 days after membrane disappeared	2 B	+	–	+	4.3–4.5	1 B	+	–	+	4.3–4.5
	4 Vir	+	–	+	4.3–4.5	3 Vir	+	–	–	4.3–4.5
	1 Vir	–	–	+	4.3–4.5					

	Case III					Case IV				
5 days before exposure	1 B	+	–	–	4.3–4.5	0 B				
	1 B	+	–	–	5.0–5.3	3 Vir	+	–	–	4.3–4.5
	1 Vir	+	–	+	4.3–4.5					
	4 Vir	+	–	–	4.3–4.5					
Same day B. Diphtheria were culture from throat	7 B	+	–	+	5.0–5.3	6 B	+	–	+	5.0–5.3
						1 B	+	–	–	5.0–5.3
						0 Vir				
14 days after membrane disappeared	1 B	+	–	–	4.3–4.5	0 B				
	2 Vir	+	–	+	4.3–4.5	2 Vir	–	–	–	4.3–4.5
	3 Vir	+	–	–	4.3–4.5	4 Vir	+	–	–	4.3–4.5

B = Beta hemolytic streptococci
Vir = Viridans streptococci

BIBLIOGRAPHY

[1]Arnold, L.: Jour. Lab. and Clin. Med., 1920, v, 587, 591.
Ibid., 1921, vi, 312.
[2]Avery, O. T. and Cullen, G. T.: Jour., Exp. Med., 1919, xxix, 215.
[3]Bernheim, J.: Ztschr. f. Hyg., 1894, xviii, 529.
[4]Ladendorff, H.: Ztschr. f. Infektionsk. etc., 1921, xxii, 151.
[5]Le Gros, Felix-Louis: Monographie des Streptocoques et des agents des septicemies metadiphteriques, etc., Paris, 1902.
[6]Loeffler: Mitteilungen aus dem Kaiserl., Gesundheitsamt, 1884, ii.

DETERIORATION OF PROCAIN SOLUTIONS*

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INTRODUCTION

THE extensive use of procain (novocain) as a local anesthetic and as an adjuvant to general anesthesia in major operations warrants an accurate knowledge of any variations in the properties of its solutions when kept over any period of time. It is frequently observed that solutions gradually develop a yellow tinge which deepens with time and is accompanied by a light flocculent sediment usually appearing at first as little tufts or balls, and later assuming a gelatinous appearance; the question immediately arises as to the safe use of such preparations. We inquire therefore:

1. Have there occurred any changes in the anesthetic properties?
2. Has the solution been rendered more, or less, toxic?
3. Is there an increase in the irritability of the preparation?
4. What is the nature of the change in color? May it be an index of a change in the irritability, toxicity, or anesthetic properties?
5. What is the nature of the sediment?
6. Is there any change in the chemical reaction to litmus?
7. Finally, what factors may be responsible for these changes, and how may they be abated?

In this work no investigation of chemical changes was made.

METHODS AND OBSERVATIONS

One per cent and ten per cent solutions in 0.73 per cent saline were placed in chemically clean bottles as follows: (a) 100 c.c. glass-stoppered bottles, exposed to light. (b) 100 c.c. glass-stoppered bottles, kept in dark. (c) 200 c.c. cork-stoppered Pyrex flasks, exposed to light. (d) 10 c.c. glass (soft) ampules, sealed by heat. A separate ampule was used for each series of determinations. These were the stock solutions with which the repeated examinations were made on the following day intervals: 1, 2, 4, 8, 15, 30, 60, 120, 270. At each determination a freshly prepared 1 per cent solution of procain in 0.73 per cent sodium chloride was used as control.

1. *Anesthetic efficiency.*—Technic of Sollmann† in determining the anesthetic efficiency on motor nerves of the frog was employed. Motor nerves were used because, although more resistant to anesthetic action, they are more reliable than the sensory in their results. The method may be sum-

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†Sollmann, T.: Jour. Phar. and Exp. Ther., 1917, x, 379.

marized briefly as follows: Muscle-nerve preparations were made from the frog's leg to include the lower part of the leg from the knee down, and the entire sciatic nerve from the knee to the spinal cord with a bit of bone attached. The entire nerve was immersed in the anesthetic solution, contained in a little trough cut in a block of paraffin. The excitability was tested with the platinum electrodes of a Harvard induction coil of constant current, applied at the distal end of the nerve at five-minute intervals. The normal efficiency (time required for a freshly prepared 1 per cent solution to cause paralysis) was taken as unity. Any variations in time greater or less than this normal are expressed in fractions of normal efficiency. For example: If the normal requires 15 minutes for complete paralysis, and a specific preparation requires 20 minutes, the efficiency of the latter is 15 divided by 20, or 0.75. In these studies the 10 per cent solutions were accurately diluted to 1 per cent before being applied to the nerve-muscle preparations. Table I shows the results of these observations. Practically no change in anesthetic efficiency occurred in these preparations over a period of nine months, except in the sealed ampules (soft glass) where the efficiency appeared immediately slightly diminished, then remained fairly constant throughout the remainder of the period. Light, darkness, and degree of concentration appeared to have practically no influence on the anesthetic activities of the preparations.

TABLE I

ANESTHETIC EFFICIENCY — NORMAL 1

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	1.	1.	1.	0.7	1.	0.8	0.8	1.	0.8
" —Dark	1.	1.3	1.		1.	1.	0.8	1.2	1.
" —Flask	1.	1.	1.	0.7	0.7	0.9	1.	1.2	1.
" —Ampule	0.7		0.6		0.7		1.	0.8	0.8
10% Procain—Light	1.	1.	1.	0.8	0.7	0.8	0.8	1.2	0.8
" —Dark	0.9	1.2	0.9	0.9	0.9	0.9	1.	0.8	0.8
" —Flask	0.9	0.8	1.	0.8	0.8	0.8	1.	1.2	0.9
" —Ampule	0.8		0.8		0.8		1.	0.8	0.8

2. *Toxicity.*—The toxicity was determined by the minimum fatal dose in white mice, injected hypodermically near the base of the tail. Several determinations showed that the toxicity of a 1 per cent solution (freshly prepared) is fairly constant, the minimum fatal dose being eight-tenths mg. of the solid drug per gram weight of the animal. In Table II are shown the minimum lethal doses in terms of milligrams of drug per gram weight of animal. It will be noticed here that there is no distinct change in toxicity that could not be ascribed to variations in the susceptibility of the animals, except in the case of the 10 per cent flask the toxicity of which increased until it doubled. Of this more will be said presently.

3. *Change in Color.*—The most conspicuous change observed was the gradual development of a yellow color in the originally colorless solutions. The degree in depth of color developed was determined by comparison with an empirically prepared caramel standard, "1" being the faintest observable, "100" containing one hundred times as much caramel as "1." These stand-

TABLE II

TOXICITY (WHITE MICE)

MINIMUM FATAL DOSE IN TERMS OF MILLIGRAMS PER GRAM WEIGHT OF ANIMAL

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	0.8				0.8	0.8	1.	1.	0.7
" —Dark	0.8				0.7	0.6	0.8	0.8	1.
" —Flask	0.8		0.8	0.6	0.6	0.6	0.8	0.9	0.8
" —Ampule	0.7		0.7		0.6		0.6	0.6	0.6
10% Procain—Light	0.8			0.8	0.8	1.	0.8	0.8	1.
" —Dark	0.8			1.	0.8	1.	1.2	1.	0.8
" —Flask	0.8	0.8	0.7	0.8	0.7	0.6	0.5	0.4	0.4
" —Ampule	0.8		0.9		0.8		0.7	0.6	0.7

ards were made up but once, and kept as permanent standards for comparison. Table III shows the results. It will be observed that at the end of four months there developed in some of the solutions a brown tinge which somewhat obscured the readings of the caramel standard. These color changes, as indicated by comparing Table III with Tables I and II, has apparently no relation to the anesthetic and toxic properties of the solutions, with the exception of the 10 per cent flask. Here we find a marked color change, with a doubling of the toxicity. The stopper of this flask was accidentally removed for a few days during the first month of the test. Increased contamination from the dust of the room may be offered as an explanation for this toxicity, since it is to be noted that the sealed ampules showed very slight development of color.

TABLE III

CHANGES IN DEPTH OF COLOR—YELLOW

NAME OF PREPARATION	AGE OF SOLUTIONS—IN DAYS								
	1	2	4	8	15	30	60	120	270
1% Procain—Light	0	0	0	1	2	6	30	30	30*
" —Dark	0	0	0	1	1	6	8	8	24
" —Flask	0	0	0	2	3	8	24	24*	24*
" —Ampule			1		2		1	1	150*
10% Procain—Light	1		1	5	5	5	8	16	2
" —Dark	1		1	4	5	5	8	16	200*
" —Flask	1		1	5	5	6	10	24*	300*
" —Ampule			5		4		4	5	5

*These solutions developed a brown tinge which obscured the yellow color. The depth of the tinge was about proportionate to the figures above indicated.

4. *Irritability.*—The irritability was determined by dropping 1 per cent concentrations of the solutions into the eye of a rabbit, and confirmed like solutions into the human eye. At no time did the rabbit's eye show appreciable irritation. The human eye was distinctly irritated by all the solutions that had developed the brown tinge, but not by the others.

5. *Appearance and Nature of the Sediment.*—After standing four days at room temperature a very faint flocculent sediment, not unlike tiny tufts of cotton less than one centimeter in diameter, appeared in all the bottles, but none whatever in any of the ampules. The tufts were distinctly smaller in the 10 per cent solutions. This sediment increased gradually in quantity and assumed a rather gelatinous appearance. When removed with a wire loop the mass clung together, and reminded one of "mother of vinegar."

Microscopic examination showed the mass to be the mycelia of common bread mold. To determine whether or not the bread mold was alone responsible for this sediment, four hard glass Florence flasks containing 1 per cent procain in 0.73 per cent saline were prepared, for comparison, as follows: The contents of three of the flasks were sterilized by boiling five minutes on three successive days, and making up the loss with sterile distilled water. One flask was stoppered with sterile plugging cotton, one with cork, and one with a rubber stopper. The fourth flask was prepared by adding to a flask of sterile 0.73 per cent saline sufficient procain to make 1 per cent (weighed and added under aseptic precautions), and stoppered with a sterile rubber stopper. Over a period of six months, at room temperature, there appeared no sediment in the three flasks. The color changes were a distinct stair-step phenomenon, the solution in the cotton-stoppered flask was a decided yellow, in the rubber-stoppered flask only a faint yellow tinge, and in the cork-stoppered flask intermediate. There was no suggestion of a brown coloration, even after a period of twelve months. There was no change in the anesthetic, toxic, and irritability characteristics of the solutions as determined by the methods outlined heretofore. The fourth flask showed the characteristic sediment of bread-mold in a coffee brown solution. This brown solution was distinctly irritating, and slightly more toxic than the other preparations. These observations indicate to us that the sediment is due to the growth of "molds" in the solution. These molds have apparently no influence on the irritability, toxicity, or anesthetic properties of the solutions until the appearance of the distinctly brown coloration. Moreover, the development of yellow color appears to be due to some oxidative process, as the greater the access of air (example: the cotton-stoppered flask) the deeper the color change, while with the almost total occlusion of air (see ampule, Table III—Color change) there was practically no change in color.

6. *Reaction.*—All the preparations, with the exception of the ampules, were faintly acid to litmus until the development of the brown coloration, when the reaction changed slowly from faintly acid to faintly alkaline. The ampules were uniformly very faintly acid or neutral.

CONCLUSIONS

1. There is practically no deterioration in the anesthetic properties of procain solutions standing over a period of several months.

2. There is no increase in toxicity or irritability except in case of gross contamination.

3. The yellow color change is no index of a change in anesthetic, irritating, or toxic properties. The development of this yellow color appears to be due to an oxidative process.

4. The sediment in procain solutions may be due to the growth of molds. Their spores may be present in the original preparations. They indicate "contamination" long before an increase in irritability and toxicity develops.

5. Procain solutions are therefore stable. If kept sterile and out of contact with air they will remain unchanged indefinitely.

LABORATORY METHODS

A SIMPLIFIED TECHNIC FOR CLINICAL BLOOD CHEMISTRY*

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THANKS to the splendid work of Folin, Van Slyke, Marshal, Benedict and others, the chemical analysis of the blood, or, as it is called more briefly but less correctly, blood chemistry is no longer today what it used to be 15 or 20 years ago. Much less time is consumed and much less blood is required, and the results are more accurate and dependable. Blood chemistry is now part of the routine work in most hospital laboratories and is of recognized value both in medical and surgical treatment.

In view of the growing popularity of blood chemistry, it appeared to us that the technic could be simplified still more. In fact, simplification is anything but a freak when it is realized that in the Beth Israel Hospital Laboratory, where our work has been carried out, from twelve to fifteen specimens are analyzed on the so-called "blood chemistry days," and the routine analysis includes CO_2 combining power, urea nitrogen, nonprotein nitrogen, creatinin, uric acid, cholesterol and sugar. The accuracy was by no means sacrificed for the sake of simplification, as several hundreds of comparative tests showed, when the standard and the simplified technic were used simultaneously. Several details appeared to us entirely unnecessary, although they are generally accepted as essential parts of the method.

We could not see the necessity of adding potassium sulphate and copper sulphate when determining nonprotein nitrogen. They are necessary in the original Kjeldahl process, when much larger quantities are used for analysis. There, they shorten considerably the time of reaction. But we don't need catalyzers or temperature raisers for the destruction of the minute quantities of nonprotein nitrogenous compounds which are present in about ½ c.c. of blood. Potassium sulphate and copper sulphate may contain nitrogen and thus become a source of error. It is true that the error is small, as only very small quantities of both substances are used, but the larger the number of substances used in the analytical process, the larger the sum of errors.

To our mind, an ideal method is one which calls for a minimum number of reagents, a minimum number of glassware and a minimum amount of manipulation. We had all these three requirements in mind when we were developing our simplified technic.

Whatever the number of reagents, their purity, at least their freedom

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from substances which may affect the accuracy, is of the utmost importance. This is obvious in the case of the determination of urea and nonprotein nitrogen as ammonium salts may occur in many inorganic compounds.

We use the following substances in the determination of urea and nonprotein nitrogen: Hydrochloric acid, sulphuric acid, sodium tungstate, sodium hydroxide, potassium hydroxide, hydrogen peroxide, potassium iodide and mercuric iodide. We found that all of them will give often a distinct reaction with Nessler's solution, sufficient to affect materially the result of the analysis, despite the fact that they are labelled C.P.

It would be unfair to call it a misrepresentation, as the amount of ammonium salts is entirely negligible for the many purposes for which the C.P. chemicals are prepared; purposes which do not include blood chemistry. The requirements of blood chemistry are too small to justify an additional outlay in getting the chemicals used in the blood analytical process absolutely free from ammonia. We therefore do our own purifying of the reagents, with the exception of sulphuric acid since we don't know of any way of freeing the latter from ammonium salts. But, whether the reagents be free from nitrogen or contaminated with the latter, a blank test must always be made along with the determination of the constituents of the blood. This is to ascertain to what extent the reagents are free from contaminations affecting the accuracy of the analysis. The freedom should never be taken as a matter of course.

The blood chemistry is being carried out at present by so-called "technicians," i.e., persons with very meagre chemical knowledge or none at all. It is therefore important to simplify the technic from one side and from the other side to emphasize many details with which every analytical chemist is familiar and which are indispensable when accurate results are to be expected.

Among these details are scrupulous cleanliness and careful, intelligent preparing and handling of the standard solutions. It is not so important for a technician to know just how many methods there are for the quantitative determinations of sugar in blood and what are the respective advantages of each one, as it is to know how to carry out a test according to one with least expenditure of time and the greatest accuracy; the latter from the clinician's standpoint.

Blood chemistry is, after all, nothing but a part of the analytical chemistry and the laws and rules of the latter are applicable to it also. Whether the ammonia, formed as a result of saponification of urea or destruction of the nonprotein nitrogen constituents of the blood, is distilled over or aerated, or whether either operation is omitted and the Nesslerization is carried out directly, is not so important as whether all the requirements of quantitative analysis have been complied with.

Duboscq's colorimeter may have certain advantages over Hellige's or vice versa, but the accuracy of the results depends to a much larger extent upon the purity of the chemicals used as standards than upon the kind of colorimeter. The testing of ammonium sulphate, potassium dichromate and

other standard reagents for their purity should be known to every blood chemistry worker.

Until the advent of the urease method, several methods of urea determination were in vogue, Folin's method, decomposition of urea with sodium hypobromite, Bang's, etc. The second, the hypobromite method, was notoriously incorrect; the first was capable of yielding correct results. Yet all the methods of urea determination got into disrepute and many advocated instead, the nonprotein nitrogen determination because the quantitative analysis of blood was done chiefly by persons not skilled in chemistry and a perfectly good method was full of traps for them.

This must be outspoken because the blood chemistry is a comparatively new thing with a big future; its field is not the chemical but the hospital laboratory, its cultivators are not the professors of pathology and biochemistry but clinicians, whose training was accomplished along different lines from those of chemistry, and their assistants, the so-called technicians. They should, therefore, be supplied with a technic which is simple and at the same time does not assume too much in the matter of fact.

Our article is a feeble effort in that direction, and at the same time, an effort towards standardization of the technic, elimination of the many methods and general acceptance of one. It would be no exaggeration to say that every hospital laboratory uses a different method of blood chemistry. This allows of no comparison between the findings of two institutions on the same subject.

To Folin, we owe an accurate method of determining urea, nonprotein nitrogen, uric acid, sugar and creatinin in 5 c.c. of blood. Many dreadful things were said against the microchemical methods and many lances were broken for the macrochemical ones. Yet no amount of praise could save the latter. Blood is not urine, and it is desirable to take as little blood from the patient as possible. It is the more desirable, when the blood taking has to be done repeatedly. It is of no use to take 25 c.c. when 5 c.c. will suffice. Sometimes, it is difficult to get more than a small volume. This applies to adults as well as to children. An analysis may go wrong, and it is necessary to repeat it, or it may be desirable to check up results.

We have found that 7 c.c. of well-oxalated blood are sufficient for an analysis which includes CO_2 combining power, urea, nonprotein nitrogen, creatinin, uric acid, cholesterol, sugar and chlorides. By well oxalated blood, we understand blood which is free from clots. It is obtained not by using plenty of oxalate, but by shaking the blood thoroughly immediately after it has been poured into the tube. As to the amount of oxalate to be used in order to prevent coagulation, we have found that 0.05 gms. is quite sufficient for 5-15 c.c. of blood. It is very convenient to introduce 1 c.c. of a solution of potassium oxalate into a test tube, to allow the liquid to wet the inside of the tube and dry it in the sterilizer. But, even with such a "prepared" tube, the shaking of the latter immediately after it has been filled with blood must not be omitted.

The technician has to analyze very often several specimens of blood

and when a more or less complete examination is required, it is desirable to do it according to a certain system which eliminates waste of time and with the smallest amount of equipment. In our work, we use nothing but glass tubes, plain and calibrated, the calibration being done by ourselves. No flasks, measuring or otherwise, funnels plain or separatory, are used. With one exception, the filtering is done away with. Whole blood is used in every test. Only in the case of CO_2 combining power do we use the oxalated plasma because the whole blood would smear the apparatus and make cleaning difficult.

We begin the analysis of several specimens by arranging eight rows of glass tubes. The first row, which we will call A, contains calibrated tubes marked 5, 10, and 15 c.c. The second row, B, contains calibrated tubes with one 10 c.c. mark. The third and fifth rows, C and E, are made up of plain test tubes, not necessarily dry, and the fourth, sixth and eighth rows, D, F, and H contain plain dry test tubes. The seventh row, G, contains dry test tubes calibrated to the 25 c.c. mark.

All the tubes are washed with an alkaline soap solution and thoroughly rinsed, first with tap and then with distilled water.

Blood specimens are centrifuged for about 8 to 10 minutes. Two c.c. of the clear oxalated plasma are pipetted off from each specimen and transferred to the tubes in row G which are then closed with cotton plugs and put away in the ice box until such time as one is ready to start with the determination of CO_2 combining power and cholesterol. The remaining 5 c.c. of blood are distributed between rows A and B, A getting 3 c.c. and B 2 c.c. The 3 c.c. are for the determination of urea, nonprotein nitrogen and uric acid. The 2 c.c. are for the determination of creatinin, sugar and chlorides.

The number of tubes in each row (row A excepted) is equal to the number of specimens to be analyzed. Row A has an extra tube which serves to control the efficiency of the urease solution and is therefore marked K.

To each blood-containing test tube in row A, we add 2 c.c. of water. Tube K gets 4 c.c. of water and 1 c.c. of a standard urea solution (1 gm. urea in 1000 c.c. of distilled water). We add to each tube 1 c.c. of urease solution prepared in the following way: 1 c.c. of permutit is washed once with 3 c.c. of 2 per cent acetic acid, and three times with distilled water, using 15 c.c. each time. The washing is done most conveniently in a capacious centrifuge tube, the water being decanted each time. Add 20 c.c. of 14 per cent alcohol, 3 c.c. of 95 per cent alcohol, 17 c.c. of water and close with a clean stopper. Shake gently for about 5 minutes, then centrifuge. This is, in the main features, Folin's proposed method for the preparation of urease solution as described by him in the *Journal of Biological Chemistry*, (1916, xxvi, 501 and 1919, xxxviii, 111).

We only reduced the quantities to one-fifth. The advantage is twofold. We avoid filtering and can use freshly prepared solutions since the preparation of the latter takes about 15 minutes.

After the urease has been added to each of the tubes in row A, the rack is put into a water-bath which has a temperature of 50° C. and kept

there for about five or ten minutes. It is very important not to let the temperature rise over 50° C. An appreciable coagulation of the protein can be noticed at even 55° C. and it increases as the temperature rises. The coagulation prevents part of the urea from being acted upon by the urease.

The rack is now taken out of the water-bath and allowed to cool. We turn our attention to row B, each tube of which we fill with distilled water up to the mark. The water is allowed to run along the walls of the tube in order to avoid foaming as much as possible. When foam is present, the addition of water should stop when the liquid is just underneath the meniscus. A drop or two of ether, amyl alcohol, or grain alcohol is added and this is sufficient to break up whatever foam there is on the surface. The tube is now filled up to the mark with a few more drops of distilled water and a pinch of dry picric acid is added. With a clean glass rod, we work the picric acid into the diluted blood until the liquid has assumed a dirty yellow color. We take out the glass rods and allow the tubes to stand a while.

The tubes in row A have been cooled down to room temperature. We add to each one 4.5 c.c. of 10 per cent sodium tungstate and fill up to the mark with 2/3 N. sulphuric acid observing the same precautions to avoid foaming and using the same means to break up the foam as in the case of row B. Folin uses 5 c.c. of sodium tungstate and 5 c.c. of 2/3 N. sulphuric acid in order to deproteinize 5 c.c. of blood. We found this proportion insufficient when whole blood is used. The 2/3 N. sulphuric acid is about 3.3 per cent and can be prepared approximately by adding slowly 18 c.c. of concentrated sulphuric acid to about 800 c.c. of distilled water in a 1000 c.c. volumetric flask and making up to the mark.

The liquids in the tubes of row A are stirred with clean glass rods until the red color of the blood has turned to dirty brown. The rods are now taken out and the tubes in both rows centrifuged for 10 to 15 minutes. While the tubes are being centrifuged, the apparatus for aeration is set up. It is made up of a copper rack with two rows of test tubes. The test tubes are of heavy glass. Those of one row are graduated into 1 c.c. divisions up to 100 c.c.; those of the opposite row are plain. Each tube is equipped with a double perforated rubber stopper with two glass tubes. One ends ½ inch underneath the stopper, the other one ¼ inch above the bottom of the test tube. Both protrude about two inches above the stopper. When making the holes in the stopper, one has to use a borer which is a size smaller than the tube, so that the latter will pass with some difficulty. (A clean, smooth hole in a rubber stopper is made easily by wetting the borer with 5 per cent lye and holding the stopper firmly against a solid surface while boring.) As soon as the tube begins to move loosely through the stopper, the latter is to be rejected.

Each row begins with a short glass tube and ends with a long one, and vice versa. Each short glass tube of one row is faced by a similar one in the opposite row and vice versa.

The test tubes of both rows and the glass tubes are carefully washed with soap and rinsed thoroughly first with tap and then with distilled

water. The graduated tubes are now filled with 10 c.c. of approximately N/30 hydrochloric acid (997 of distilled water, 3 c.c. of fuming hydrochloric acid) and closed tightly with the rubber stoppers and the rubber tubes attached. The plain tubes are loosely closed with the rubber stoppers in such a way that the short glass tubes are outside of the test tubes.

Having proceeded thus far with the preparations for aeration, we now start with the oxidation of the nonprotein nitrogenous constituents of the blood. We set up a row of tiny Erlenmeyer flasks, 30 to 40 c.c. in capacity. We draw off with a clean dry pipette 8 c.c. of the clear supernatant liquid from each of the tubes of row A and run 3 c.c. into each of the tiny Erlenmeyer flasks and 5 c.c. into each of the tubes of row E. Another 3 c.c. portion of the filtrates, which may be turbid, is introduced into each of the plain aeration tubes marked "U." We then take an extra Erlenmeyer flask and run in 0.9 c.c. of 10 per cent sodium tungstate and 0.9 c.c. of 2/3 N. sulphuric acid of urease and 1 c.c. of distilled water. This flask will serve as a blank test, that is, it will indicate how much nitrogen there is in all the quantities of the chemicals used in the determination of urea N. and nonprotein nitrogen.

It may appear that it would be a much simpler procedure to determine once for all the degree of freedom from objectionable compounds of all the chemicals used in the determination of urea N. and nonprotein nitrogen. We can deduct the sum of the quantities of nitrogen combined in the quantities of the chemicals used in the analysis from the quantity of the found nonprotein nitrogen and thus eliminate the error due to contamination. Yet this would not be correct. Occasional carelessness on the part of the operator, or tampering with by unqualified persons may bring about a contamination after the chemicals have been tested and the degree of their purity, so far as the nitrogen is concerned, duly determined. We cannot say enough to emphasize the importance of a blank test in microchemical blood analysis. We will go so far as to declare as doubtful any analysis where a blank test has been omitted. The use of a blank test where it has been previously omitted, will sometimes produce very curious results. It will bring high urea nitrogen and nonprotein nitrogen figures where they are inexplicable within acknowledgingly normal limits and eliminate a good deal of speculation.

Some observers found that normal persons may have as much as 27 mg. of urea nitrogen in 100 c.c. of blood. It would be curious to know whether they used a blank test and in what other way they made it certain that all of the nitrogen they found originated in the blood.

To go back to our Erlenmeyer flasks, we run into each one ½ c.c. of concentrated sulphuric acid, put the flasks upon a sand bath and the latter upon the electric stove. The boiling proceeds very smoothly and without bumping. If there is such annoyance, the layer of sand is made a bit thicker. After the liquid has turned a black-brown, the heating is still continued for about five minutes, after which the flasks are taken off the sand bath and allowed to cool.

After the flasks have cooled, one may add to each 1 c.c. of hydrogen

peroxide if one wishes, but this is not necessary. The hydrogen peroxide merely burns off the carbon and restores to the liquid its original color, but it does not complete the conversion of the organic nitrogen into ammonia. This job is thoroughly done by sulphuric acid. The use of hydrogen peroxide would be a necessity if it were possible to Nesslerize directly without previous aeration.

Unfortunately, the direct Nesslerization fails so often or yields solutions which turn rapidly turbid, that we do not advise its use at all. To be sure, putting up the apparatus for aeration and disconnecting it after the aeration is a considerable annoyance. But an even greater annoyance is noticing how the liquid which has been Nesslerized rapidly turns turbid and a red sediment begins to fill up the tube and makes the determination impossible.

Despite numerous efforts, we are unable to explain what causes the precipitation of the red sediment. Having noticed that the direct Nesslerization of the liquid containing ammonia, as derived from a standard urea solution, incubated with urease, gave less trouble, we thought that the latter might be due to the presence of an alkali sulphate. (Alkali sulphate is formed when Nessler's reagent is added to sulphuric acid.) We added variable quantities of potassium and sodium sulphate to standard ammonium sulphate and then added Nessler's solution. We had no trouble in Nesslerizing. This shows that the alkali sulphates are not responsible for the turbidity. We tried a mixture of phosphoric and sulphuric acids as suggested by Folin. The results were not encouraging and the mixture attacked the flasks. We did not give up hope yet of finding the conditions under which a Nesslerized liquid will keep for several hours as is the case when aeration has been used. But for the present, we prefer not to take any chances with direct Nesslerization.

While the Erlenmeyer flasks are being heated on the electric stove, we may start with the determination of the CO_2 combining power. The procedure is similar to the one described by Van Slyke. Yet we thought it expedient to introduce a few changes.

Instead of separatory funnels we use plain dry test tubes (row F) large enough to accommodate a double perforated stopper. The latter has two glass tubes passing through it. One is short and ends underneath the stopper. The other one is longer and ends $\frac{1}{8}$" above the surface of the blood. Each tube is in its turn closed with the stopper, air is blown through which has previously passed a bottle filled partly with glass beads and 1 c.c. is introduced into the CO_2 apparatus which has been previously evacuated in the well-known way. For convenience, the stem to which the CO_2 apparatus is attached is equipped with three bottles having syphons. One bottle is filled with distilled water, the other one with caprylic acid, the third with 5 per cent sulphuric acid. This allows quick washing of the apparatus previous to evacuation and obviates the necessity of testing for the presence of acid with ammonia.

It is customary to manipulate the apparatus after sulphuric acid has been

added so that the mixture of blood water and sulphuric acid is finally brought to the larger knee of the CO_2 apparatus while the mercury is allowed to rush into the calibrated part through the more narrow knee. This is not necessary. There is little difference whether the gas touches mercury or the mixture of the liquids—the result is not affected by this fact materially. Why then the manipulation? Furthermore, the apparatus could be made by entirely omitting the more narrow knee and it would be less fragile and expensive.

The aeration can now be started. The contents are transferred carefully to the plain aeration tubes marked NPN, the flasks rinsed twice with 2 or 3 c.c. distilled water. Every plain tube, whether marked NPN or U gets enough sodium hydroxide (20 per cent) to produce a distinctly alkaline reaction, and enough amylalcohol to form a layer about one-fourth inch high. There is no reason to fear that a loss of liberated ammonia will follow the addition of lye if the aeration tubes are not closed quickly. We cannot see how such loss could take place, as we are using only 1.2 c.c. of blood for both urea N. and NPN determination.

When all the tubes are closed with stoppers they are connected with the suction pump and the latter turned on full. Thirty minutes are sufficient to complete the aeration. We loosen the stoppers beginning with the tube connected with the sulphuric acid bottle, letting the water run through the suction pump in the meantime. When the last stopper has been loosened, we shut off the water, pull out each stopper and rinse the long glass tube of each stopper with distilled water. We run 2 c.c. of an ammonium sulphate solution containing 0.944 gm. of $(NH_4)_2SO_4$ in 1000 c.c. water into a 25 c.c. graduated cylinder, then 10 c.c. water, then 8 c.c. diluted Nessler's solution (1 part Nessler's solution, 5 parts water). The color thus produced is compared in Hellige's colorimeter with the color obtained when the diluted Nessler's solution is added gradually to each of the tubes containing ammonium chloride and the tubes are then filled up to the 20 c.c. mark. In case of large quantity of urea in the blood, we dilute to 40, 60, 80 or 100 c.c.

Calculation with the Hellige Colorimeter.—Suppose the reading in the case of urea was 25. Multiplying this number by .0225 we find the number of mg. of nitrogen which 100 c.c. of a solution must contain in order to produce the same intensity of color. It will be 0.56 mg. It is obvious that 20 c.c. contain only $\frac{0.56 \times 20}{100} = \frac{0.56}{5}$ mg. For the estimation of urea nitrogen, we took 3 c.c. of blood diluted to 15 c.c. and then pipetted off 3 c.c. These 3 c.c. represent $\frac{3 \times 3}{15}$ or $\frac{3}{5}$ c.c. of the blood. One hundred c.c. of the blood will contain $\frac{0.56 \times 5 \times 100}{3 \times 5} = \frac{56}{3}$ mg. urea nitrogen, or urea nitrogen $= \frac{25 \times 2.75}{3} = 25 \times \frac{3}{4}$

Assuming that:

R– Reading (Number of mg. corresponds to number read on scale of colorimeter.)
A– Number of c.c. of blood taken for analysis
V– Volume to which the blood was diluted
b– The number of aerated and subsequently Nesslerized c.c. of clear filtrate
V_1– Volume to which the Nesslerized solution was diluted

we get the following general formula:

$$\text{Urea N} = \frac{R \times .0225 \times V \times 100 \times V_1}{ab \times 100} =$$

$$\frac{R \times .0225 \times V \times V_1}{ab}$$

The same formula applies to the calculation of nonprotein nitrogen.

We are now ready to determine glucose, creatinin and the chlorides.

We draw off about 5.5 c.c. of the supernatant yellow liquid from each of the tubes in row B and run in 2 c.c. into each of the tubes in row D, 3 c.c. into each of the Erlenmeyer flasks in row K and 0.4 c.c. into each of the tubes of row E. We add to each tube in row D 0.1 c.c. of 10 per cent NaOH or, still better, 0.1 c.c. of 2 N. sodium hydroxide. We mix well and allow ten minutes for the reaction between the creatinin, sodium hydroxide and picric acid. The reaction is supposed to be a reduction, the picric being reduced in alkaline solution to picramic acid. It is also possible that some kind of an azo-oxy compound is formed. Since azo-oxy compounds are not very stable and change readily into azo compounds, it is not recommended to wait longer than 10 to 15 minutes. During this time, we add ½ c.c. of 10 per cent sodium carbonate to each of the test tubes in row C and heat. The yellow liquid begins to turn red. When there is no more perceptible change in color, the heating is discontinued.

We now fill up the wedge of Hellige's colorimeter with a N/4 solution of potassium dichromate (12.44 gm. in 1000 c.c.), pour successively the contents of each of the tubes of row D into the small cup of the colorimeter and take a reading.

Calculation.—The number of mg. of creatinin per 100 c.c. of blood is found by multiplying the number found through matching the standard with the unknown by 5 (as the blood has been diluted 5 times) and 100.

The contents of each of the tubes in row C we pour successively into the graduated tube of Kuttner's colorimeter, rinse the tube out a few times with a few drops of water, and then add so much water until the color matches with that of either standard A or B (the latter is twice as strong as A). The mark on the graduated tube to which the liquid had to be diluted gives automatically the number of mg. of glucose per 100 c.c. of blood if A has been used. When B is used, we multiply the number by 2. When strongly diabetic blood is to be used, we use 0.2 c.c. instead of 0.4 c.c. of the yellow liquid.

We found Kuttner's colorimeter as reliable in *clinical* work as other colorimeters, and a good deal more convenient in view of the minute quantities of blood and little time required to carry out its determination. We wish

to state, however, that the blood should be drawn from an arm vein and not by finger puncture to obviate dilution by tissue juices.

Chlorides.—To each Erlenmeyer flask of row K, we add 10 c.c. of n/50 silver nitrate, which also contains ferric ammonium sulphate. (This ammonium sulphate-silver nitrate solution is best prepared by dissolving 2.158 gm. of silver nitrate in 600 c.c. of distilled water contained in a 1000 c.c. measuring flask, adding a solution of 100 gm. ferric ammonium sulphate in 100 c.c. of 25 per cent nitric acid which have been previously boiled in order to expel the nitrous acid, and then filling up to the mark with distilled water.) The contents of the Erlenmeyer flasks are stirred well and heated. The silver chloride will coagulate and will not interfere with the further course of the analysis. We now add 5 c.c. of distilled water and titrate with n/50 ammonium sulphocyanate. When all the silver has been precipitated as insoluble silver sulphocyanate, the reaction between ammonium sulphocyanate and the ferric salt, characterized by a red color, will take place.

Calculation.—Suppose the excess of silver nitrate was 7. This means that 3 c.c. of n/10 silver nitrate were necessary to precipitate the sodium chloride present and that the 3 c.c. which we took for the determination of the chlorides contained as much sodium chloride as there is in 3 c.c. of a n/50 sodium chloride solution. n/50 sodium chloride solution contains 1.169 gm. per liter or 1.17 mg. in 1. c.c.. Three c.c. will contain 3.51 mg. of sodium chloride, and 1 c.c. of blood will contain $3.51 \times \frac{10}{3.2}$. One hundred c.c. will contain $3.51 \times \frac{10}{3.2} \times 100 = .585$ mg. of sodium chloride.

$$\text{Sodium chloride (100 c.c. of blood)} = (10-7) \times 1.17 \times \frac{10}{2 \times 3} \times 100$$

If we put $7 = a$
$3 = b$
$2 = c$

we obtain the following general formula:

$$\text{NaCl (100 c.c. blood)} = (10-a) \times \frac{1170}{bc}$$

Uric Acid.—Into each of the tubes in row E, we run 2 c.c. of a 5 per cent silver lactate solution in 5 per cent lactic acid. This precipitates with uric acid as silver ureate. The tubes are now centrifuged for about four minutes, the clear supernatant liquid poured off, 5 c.c. of water added, the precipitate stirred up, the centrifugation and the subsequent decantation repeated. We now add just enough of a 5 per cent sodium cyanide solution to dissolve the precipitate. We transfer the liquid to a 10 c.c. graduated cylinder, wash out twice with a few c.c. of a saturated sodium carbonate solution (225 gm. in 1000 c.c. of water). We now prepare the standard by running into a 50 c.c. volumetric flask 10 c.c. of the standard uric acid solution, prepared according to Folin. (1 gm. of uric acid dissolved in 500 c.c. of water with the aid of 1 gm. lithium carbonate; 50 c.c. are transferred to a 1000 c.c. measuring flask, 500 c.c. of a 20 per cent solution of sodium sulphite are added, the flask is filled up to the mark and the uric acid solution

transferred to 200 c.c. bottles which are kept tightly stoppered.) The 10 c.c. of standard uric acid solution represents 1 mg. of uric acid. We now add 20 c.c. of saturated sodium carbonate solution and 1 c.c. of uric acid reagent, prepared according to Folin, fill up to the mark with distilled water, mix well and run the blue liquid into the wedge of Hellige's colorimeter. To the liquid in the 10 c.c. graduated cylinder, we add ½ c.c. of uric acid reagent, fill up to 5 c.c. and mix well before taking reading.

Calculation—

$$\text{U.A. (100 c.c. blood)} = \frac{R \times V \times V_1 \times 100}{ab \times 100} = \frac{R \times V \times V_1}{ab}$$

R = Reading (By reading we understand the number of mg. corresponding to the number read on the scale of the colorimeter.)
V = Volume to which blood has been diluted
V_1 = Volume to which blue liquid has been diluted
a = Number of c.c. of protein free filtrate taken for uric acid determination
b = Number of c.c. of blood used for the determination of urea N., NPN, and uric acid

Like several other observers, we noticed that if the sodium carbonate solution is added before the uric acid reagent, the blue color does not appear often. We can offer no satisfactory explanation for this phenomenon.

Cholesterol.—To each of the tubes in row G, we add 10 c.c. of alcohol-ether mixture (3 parts absolute alcohol to 1 part ether) and run in ½ c.c. of blood very slowly, drop after drop, stirring well with a glass rod after every addition. We heat the tubes until the liquid begins to boil, cool to room temperature, take out the rods, rinse them with little of ether-alcohol mixture, fill up with this liquid till the mark, filter into dry clean tubes (row H), draw off 10 c.c., run into dry clean 30 c.c. beakers and evaporate the ether-alcohol mixture in a water-bath of about 85° C., taking care that the heating should be discontinued as soon as the beakers are dry. We extract the cholesterol three times with small portions of warm cholesterol (1 c.c. at a time), transferring the extract to a graduated 10 c.c. cylinder, fill up to the 1 c.c. mark with chloroform, add 2 c.c. acetic anhydride (the latter must be colorless—if it became colored, it must be rectified by using an air condenser) and one drop of concentrated sulphuric acid and shake. It is customary to put the mixture in a dark place and wait ten minutes until the full color develops, which is then compared with a standard solution of naphthol green B. (2 c.c. of a 0.1 per cent aqueous of the dye with 17 c.c. of water). The 0.1 per cent solution is good for about a month, after which time it should be rejected and a fresh one prepared. When freshly prepared, it should be checked up by a standard cholesterol solution, although the necessary correction is mostly within the limits of permissible error.

Calculation —

$$\text{Cholesterol (100 c.c. blood)} = \frac{R \times V \times 100}{B \times A}$$

R– Reading
B– Amount of blood used
V– Volume to which the alcohol-ether extract has been diluted
A– Amount of alcohol-ether extract taken for determination.

We wish to thank Dr. Max Kahn for his friendly advice during the course of these experiments.

A SUCCESSFUL METHOD FOR THE ISOLATION OF DIPHTHERIA BACILLI FROM MIXED CULTURES*

By Anna I. van Saun and Isabelle A. Graves

IT would seem as though the last word must have been said long ago with regard to cultural work on diphtheria. Conditions vary so in different places however, that methods which are excellent for one laboratory are quite impossible for another and it has occurred to us that the results of our experiences with various procedures for use in the isolation of the diphtheria bacillus when neither ascitic fluid nor blood could easily be obtained, might be helpful to other laboratories in the same position.

We communicated with a number of laboratories as to methods used and discovered that several of them varied as to their plating medium.

One large State Laboratory, that of New York, plates the original culture on dextrose serum agar and also uses ascitic broth for enrichment. Fishings are made to Loeffler's. This method has been in use for a number of years in the Research Laboratory of the Department of Health of New York City and was first suggested by Dr. Anna Williams. We also used this method whenever we could obtain ascitic fluid. It gives excellent results.

The State Laboratory of Connecticut has followed the above method whenever ascitic fluid could be obtained.

The State Laboratory of Massachusetts has used large tubes of Loeffler's blood serum for the isolation of the diphtheria bacillus, making a number of transplants from the original culture and fishing with the aid of a hand lens from typical colonies. The director states that good results have been secured by the use of this method.

The Laboratory of the City of Chicago sends out an outfit consisting of a small aluminum box filled with Loeffler's blood serum which really constitutes a small plate and with which good results are said to be obtained. We have not personally tried this method owing to considerable difficulty experienced in handling the boxes.

The Laboratory of the City of Bridgeport uses tubes of Loeffler's in the manner of the State Laboratory of Massachusetts and fishes to broth. The Director of the laboratory states that the method is not successful with contaminated or overgrown cultures. We have not been successful in growing the diphtheria bacillus in plain broth and therefore did not try the above method.

The Bender Hygienic Laboratory of Albany, N. Y., plates the original culture on blood agar, fishing from thence to Loeffler's blood serum. The Director informs us that the results are excellent. We were not able to use this method on account of the difficulty in obtaining blood. Horse serum agar was

*From the Bureau of Laboratories, Department of Health, New Haven, Connecticut.
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suggested but it was found that the cost of sterile horse serum to any but antitoxin laboratories would be prohibitive.

It seemed to us that the method used by Miss Beckler of the State Laboratory of Massachusetts was the simplest of those noted above and we tried it, but our experience with it led us to believe that a larger inoculation surface would be an improvement, especially with badly contaminated or overgrown primary cultures. We therefore experimented with Loeffler's blood serum in Petri dishes, finally securing plates which gave excellent results with 90 per cent of our cultures. The laboratory runs a census of about twelve thousand (12,000) diphtheria cultures annually—5765 were received from January 1, 1922 to July 1, 1922. One hundred and forty-five (145) cultures were selected from this number for isolation.

Any good formula for Loeffler's blood serum may be used. We fill the plates to about an eighth of an inch in depth with the mixture and bring the medium rather rapidly to the coagulation point in the autoclave with the door about three-quarters closed. This takes four or five hours. Then the door is completely closed and steam allowed to escape through the valve for about fifteen minutes, after which the valve is closed and the plates are autoclaved for 20 minutes at 15 pounds pressure when they are ready for use. They frequently contain a large amount of water of condensation and this is poured out just before they are inoculated. Bubbles sometimes occur but such plates can be used. Inoculation is made with a platinum loop on the center of the first plate after which a glass spreader is used to distribute the growth on the first plate, using the same spreader to distribute the growth on two succeeding plates. Occasionally 4 plates are used for badly contaminated cultures. As a general rule we are not obliged to replate cultures. Fishings, of course, are made macroscopically to Loeffler's tubes and we find Miss Beckler's suggestion as to the hand lens very helpful.

We ran, as a comparison, duplicate cultures on ascitic agar plates. We found that as far as results went, the media were of practically equal value with perhaps some slight advantage on the side of the Loeffler's blood serum plates in the case of grossly contaminated cultures.

The method is so simple that it is quite possible that other laboratories are using it, though inquiry has not as yet elicited such information. The procedure is one which we can recommend highly to any laboratory making Loeffler's blood serum.

NOTE ON THE COMMENTS OF REZNIKOFF ON THE USE OF SODIUM SULPHATE AS A PRECIPITANT OF "PSEUDOGLOBULIN"*

By Paul E. Howe, Princeton, N. J.

In an article on "The Action of Proteins and Blood Serum on Colloidal Gold Solution and Its Quantitative Interpretation,"[1] Reznikoff makes the following statement:

"There is no chemical method for determining proteins which is accurate and practical. Howe's[2] sodium sulphate method was tested against the pure proteins to determine its applicability to these experiments. It was found that neither 14 per cent nor 22.2 per cent sodium sulphate had any effect on the albumin; that the 14 per cent salt precipitated 97.88 per cent of the euglobulin; that the 22.2 per cent salt precipitated 96.4 per cent pseudoglobulin; but that the 14 per cent solution precipitated 89.16 per cent of the pseudoglobulin, thus making the determination of the separate globulins impossible."

In his conclusions, the following occurs:

"10. Howe's method for isolating the globulins was not found to be accurate by testing it with the pure proteins used in these experiments, the euglobulin precipitating agent precipitating 89.16 per cent of the pseudoglobulin."

These statements apply only to the protein preparations used in the experiments presented in the paper. These proteins were prepared by the usual salting out procedures—saturated sodium chloride and half saturated ammonium sulphate. After salting out they "were not dialyzed. . . . *but were precipitated with 95 per cent alcohol, centrifuged, washed with 80 per cent alcohol three times, centrifuged each time, and then washed into a dish with 95 per cent alcohol. The mixture was dried by an electric fan and the globulins taken up with 0.4 per cent saline.*" Furthermore, Dr. Reznikoff states in a personal communication to me that no other globulin precipitant than sodium sulphate was tried on the preparation.

In the absence of suitable controls there is no need for a detailed discussion of the validity of the evidence against sodium sulphate, and incidentally probably all other salts, as a means of separating proteins from blood serum or plasma in general save to say that preparations of pseudoglobulin have been made in this and in another laboratory which gave little or no precipitate with 14.2 per cent sodium sulphate. While Dr. Reznikoff refers only to his particular preparations, the implication in his summary is that his preparations were above reproach and, therefore, the salting out procedure was at fault, which is not the case at all.

*From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

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In the last section of the summary the statement that, "New methods for the separation and determination of proteins are necessary before the very obvious and probably important differences of these substances in various clinical conditions can be investigated" is only in part true with regard to both the procedures and the results obtained. The necessity for further work is, however, evident to any one who has worked with proteins. Since the beginning of protein chemistry it has been necessary to resort to salting out, acidification, or dialysis, and in some cases (products of protein hydrolysis) to precipitation with alcohol to obtain various protein "fractions" of blood. Refinements have come especially in choosing a particular salt concentration, acidity, or the dilution of the blood serum or plasma, and in the methods of analysis of the fractions obtained. The advantage of sodium sulphate or other salts over ammonium sulphate, the classical precipitant, lies in the fact that the nitrogen of the protein can be determined directly. There are objections to magnesium sulphate in that, (a), solutions from protein precipitations filter slowly and, to a less extent, (b), a precipitate of magnesium hydroxide is formed when alkali is added in the Kjeldahl procedure. For those who prefer the refractometric procedure of Robertson or the colorimetric procedure of Wu the presence of ammonia is not an important factor. The latter methods of analysis, however, rest fundamentally on "salting out" processes—which the statements of Reznikoff in effect attack. It is not necessary to use sodium sulphate to precipitate the proteins in order to determine the protein nitrogen directly since sodium phosphate, potassium phosphate, or magnesium sulphate will, as we have found, give all of the usual "globulin fractions" as satisfactorily as sodium sulphate. A single precipitation with any salt will, of course, carry with it certain errors, but these are under the circumstances inevitable. We still believe that knowledge with regard to pathologic changes can be greatly enhanced by the use of the existing methods for the determination of proteins.

REFERENCES

[1]Reznikoff, Paul: Jour. Lab. and Clin. Med., 1922, viii, 92.
[2]Howe, Paul E.: Jour Biol. Chem., 1921, xlix, 93, 109.

CONCERNING THE FORMALDEHYDE-GEL REACTION IN THE DIAGNOSIS OF SYPHILIS*

By Robert A. Kilduffe, A.M., M.D., Pittsburgh, Pa.

AMONG the numerous serologic "short-cuts" proposed for the diagnosis of syphilis is the formaldehyde-gel reaction of Gate and Papacostas.[1]

As described the test consists of the addition of two drops of 40 per cent C.P. formaldehyde solution to one c.c. of clear serum, the tube, after mixing, being plugged and incubated for 24 to 48 hours.

*From the Laboratories of the Pittsburgh Hospital.
Received for publication, January 16, 1923.

A positive reaction is indicated by the formation of a jelly-like clot. In their series of 400 cases an agreement of 85 per cent was claimed with the Wassermann test and the reaction advocated as a simple and reliable means of testing for syphilis in the office of the practitioner.

The only explanation thus far advanced of the mechanism of the reaction is that of Holborrow[2] who believes that the formation of the clot is due to the direct action of the formaldehyde upon the proteins of the serum and who holds it probable that an acid protein has the ability to produce gel formation while alkali protein does not. Gel formation, therefore, he looks upon as an indication of decreased serum alkalinity without indicating its cause. He looks upon the test in the light of his findings with it with much reserve.

Sufferin[3] reports an agreement of 90 per cent with the Wassermann test but his investigations include only eleven cases which at once robs his conclusions of all significance.

Ecker[4] in a series of 500 cases found an agreement with the Wassermann of only 37 per cent and concludes that the reaction is of no diagnostic value "because of its failure to react in clinically and serologically clear-cut cases of syphilis and the occurrence of positive reactions in the absence of the disease."

Similar conclusions were reached by Armangue and Gonzales[5] and by Kingsbury[6] who emphasizes the futility of basing conclusions upon too small a number of cases, such as the series of Sufferin.

Burke[7] in a series of 319 sera obtained an agreement with the Wassermann of 85 per cent but notes the fact that the reaction is prone to give false positive and false negative results and looks upon the test as unsuited for use alone as a means of diagnosis.

In the present communication the results of a comparison of the formaldehyde-gel and Wassermann reactions are reported in a series of 480 sera.

The Wassermann reaction was made after the modification recently described by Kolmer[8] and proposed for standard adoption, experience with this technic having shown it to be remarkably delicate and reliable.[9]

The formaldehyde-gel reaction was made upon the same sera at the same time as the Wassermann.

The sera tested comprised those examined for diagnosis, those from known syphilis and from syphilis under treatment, and a variety of nonsyphilitic conditions from the wards of the Pittsburgh Hospital.

All sera were inactivated for 15 minutes at 56° C. and the majority tested within 24 hours of their receipt, none being over seventy-two hours old.

The technic of the Gate and Papacostas test consisted in the addition of two drops of 40 per cent formalin (Merck) to one c.c. of clear serum in a small tube which was then well shaken and the mixture incubated without plugging the tube in a bacteriologic incubator at 38° C. for 24 hours, after which the reading was made.

The formation of a firm, jelly-like clot was considered a positive reaction. Occasionally a membranous scum or pellicle formed on the surface adherent to the tube at its edges. On jarring the tube, however, this was dislodged show-

ing fluid serum beneath. Such sera were read as negatives. Only positive and negative readings were made, quantitative readings being impossible.

Of the 480 sera tested 80 or 16.6 per cent were Wassermann-positive in varying degree, the remainder being negative.

With the formaldehyde test 170 or 35 per cent were positive; of these only 40 or 8.3 per cent occurred with the Wassermann positive sera, the reaction being entirely negative with 50 per cent of the Wassermann-plus sera.

Of the 400 Wassermann negative sera 130 were positive to the formaldehyde reaction.

It is obvious, therefore, that the formaldehyde-gel reaction will give negative results in syphilis, and positive results in the absence of syphilis in a sufficiently high proportion of cases to render it extremely unreliable as a means of serologic diagnosis and the test should, therefore, not be used for that purpose.

REFERENCES

[1]Gate and Papacostas: Compt. rend. Soc. de Biol., November, 1920, xx, 1432.
[2]Holborrow, A. G.: Lancet, London, August 5, 1922, ii, No. 5162, p. 274.
[3]Sufferin, C.: Lancet, London, 1921, cci, 1107.
[4]Ecker, J.: Jour. Infect. Dis., 1921, xxix, 359.
[5]Armangue and Gonzales: Jour. Infect. Dis., 1922, xxx, 443.
[6]Kingsbury, W. N.: Lancet, London, 1921, cci, 779.
[7]Burke, V.: Arch. Dermat. and Syph., April, 1922, v, 4.
[8]Kolmer, J. A.: Am. Jour. Syph., June, 1922, vi, 82.
[9]Kilduffe, R. A.: Arch. Dermat. and Syph., December, 1922, vi, 709.

A METHOD FOR THE DETERMINATION OF HEMOGLOBIN*

By Russell L. Haden, M.A., M.D., Kansas City, Mo.

THE many methods and devices suggested for the estimation of hemoglobin bear witness to the unsatisfactory nature of the determination. The only direct method which is at all practical is the oxygen capacity method of Haldane as adapted by Van Slyke to his blood gas apparatus.[1] This procedure requires practice and technical skill and is too time-consuming for routine use. It should, however, be the method of choice in every case of anemia where a careful and accurate determination of the hemoglobin is of value in diagnosis or prognosis.

All the commonly used clinical methods for the determination of hemoglobin are colorimetric in nature and consequently indirect. The accuracy of the results obtained in all such methods depends upon several factors, principally the standard and the calibration of the instrument. Satisfactory results can be obtained with almost any method or instrument provided certain fundamental considerations be understood and reckoned with.

Probably the most valuable method for routine clinical use is the acid

*From the Department of Internal Medicine, University of Kansas, School of Medicine, Kansas City, Kansas.
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hematin method of Sahli. Good results will be obtained if the pipette used is accurately graduated, the comparison tubes are of equal bore, and the standard solution is correctly made. It is unusual, however, to find a Sahli instrument on the open market fulfilling these several requirements. The method is not readily adaptable to the rapid hemoglobin determination on many patients, the tubes cannot be easily transported after the blood specimen has been placed in them, and the readings need to be made at a uniform time after the specimen is obtained.

The Dare method has the advantage of a permanent standard of colored glass and the use of undiluted blood. The instrument is expensive and fragile. It can be used for no other purpose than hemoglobin estimation. Quite widely different readings are obtained with the same blood on different Dare instruments since it is almost impossible to manufacture a colored glass which will give uniform results in colorimetric procedures. It has the added disadvantage that the colors to be matched are shades of red—a color with which it is most difficult to make good colorimetric readings.

The method described below for the determination of hemoglobin has some advantages which make it of value under certain conditions. It was originally devised to supply the need for a method in which the blood specimens could be obtained at the bedside, be sent to the laboratory, and the readings be made when the cell counts were done. The blood is diluted when drawn and requires no further manipulation, the dilution is made in an ordinary red cell counting pipette, it may be easily transported without danger of loss, only a small amount of blood is required, and the determination may be made within any time from five minutes to twenty-four hours after the specimen is obtained. The ease of transportation and the fact that the readings may be made as is most convenient make the method especially valuable wherever it is desirable to obtain the specimen outside and make the determination in a central laboratory as is commonly done in many large hospitals.

The method is an adaptation of the acid hematin method of Sahli to the Hellige colorimeter (universal colorimeter of Autenrieth and Konigsberger). The use of this instrument for hemoglobin determination is of course not new, the instrument having been originally devised as a hemoglobinometer and has been used by others for a similar purpose. As ordinarily used, however, a special pipette is necessary and the blood in the unknown and in the standard are in the same dilution. If the blood is diluted 1:100 with the special pipette as is usually done, the color changes over the part of the scale most used are so slight and gradual as to make accurate readings almost impossible. We have found that by so diluting the standard as to make all readings from 30 to 110 per cent fall on the middle third of the wedge of the colorimeter exceedingly sharp color changes are obtained. In the method as given the standard is thus diluted.

In order that readings may be made on the fluid contained in the bulb of small red cell pipettes a thin piece of wood such as a tongue depressor is placed in the bottom of the cup holder. This raises the bottom of the cup on a level with the lower margin of the aperture of the colorimeter through

which the readings are made. It is unusual to find a Hellige colorimeter on which the scale is correctly placed, hence every instrument should be carefully calibrated. The accuracy of the readings will depend in large measure on the care with which the calibration is made. In the preparation of the standard solution we have taken 15.6 grams of hemoglobin per 100 c.c. as equivalent to 100 per cent.[2] The method is described in detail below.

CALIBRATION OF THE HELLIGE COLORIMETER

With the same solution in both cup and wedge read the colorimeter scale when the color of the solution in the cup matches that in the wedge. Normal blood diluted 1:100 is satisfactory for this purpose. This scale reading determines the 100 per cent mark when the unknown and the standard are equally diluted. Designate this point "Y." Now raise the wedge until its lower end is just above the lower level of the aperture through which the readings are made. This determines the point on the scale equivalent to 0 per cent. This scale reading may be designated "X." The depth of the cup at its bottom is 10 mm. It is evident that the depth of the wedge at the point at which the colors match must be 10 mm.

The percentage of substance in the cup in terms of that in the wedge is:

$$10 \times P \times B = (R-X) \times \frac{10}{Y-X} \times A \times 100$$

Where:

P is the percentage of substance in the unknown solution
A is the concentration of the standard
B is the concentration of the unknown
R is the reading of the scale at the point at which the colors match
Y is the point on the scale equivalent to 100 per cent
X is the point on the scale equivalent to 0 per cent

then

$$P = (R-X) \times \frac{100}{Y-X} \times \frac{A}{B}$$

The unknown blood is diluted 1:100 or is in a concentration of 1 per cent. For simplicity of calculation it is desirable to make the factor $\frac{100}{Y-X} \times A$ equal a whole number. If this number be 2 the readings will fall on the middle portion of the wedge when the blood is diluted 1:100. In a properly constructed wedge (Y-X) equals 80 mm., hence with the standard in a concentration of 1.6 per cent the calculation will be as follows:

$$P = 2\ (R-X)$$

Thus the percentage of hemoglobin in the specimen under consideration can be read off directly by subtracting the zero point from the scale reading and multiplying by two. It is convenient to construct a scale for each instrument showing the hemoglobin in per cent and in grams corresponding to the different scale readings.

PREPARATION OF REAGENTS

1. Tenth normal hydrochloric acid. This may be made sufficiently accurate by diluting 11.7 c.c. of concentrated hydrochloric acid with distilled water to a volume of 1,000 c.c.

2. Acid Hematin Standard. Withdraw by venipuncture about 50 c.c. of blood, defibrinate by whipping, and strain through gauze. Determine the hemoglobin content of the defibrinated blood by the oxygen capacity method in the Van Slyke apparatus.[1] Dilute the blood with N/10 hydrochloric acid to make a 20 per cent solution of blood containing 15.6 grams of hemoglobin per 100 c.c. of blood; i.e., if the blood used is found to have a hemoglobin content of 14.0 grams per 100 c.c., then 22 c.c. of the blood must be employed to make a 20 per cent solution of a blood with a hemoglobin content of 15.6 gm. per 100 c.c. Mix well and let stand for 24 hours. Add an equal volume of glycerin. Store in a glass-stoppered bottle, preferably in a cool place away from the light. This solution will keep without fading for months. It represents a 10 per cent solution of a blood containing 15.6 grams of hemoglobin per 100 c.c. From this stock solution any dilution desired, usually 1.6 per cent, may be made. Equal parts of glycerin and N/10 hydrochloric acid are employed as a diluent.

Procedure.—Draw up blood from a puncture wound in the ear or finger tip exactly to the 1 mark on a red cell counting pipette. Fill to the 101 mark with N/10 hydrochloric acid. This makes a 1:100 dilution of the blood.

Allow the preparation to stand for 10 minutes or more. At least 10 minutes is required for the conversion of the hemoglobin into acid hematin. There is very little change after this time, so the reading may be made 24 hours or even 48 hours after the dilution is made.

Blow the contents of the pipette into the cup of the colorimeter after discarding the first drop. This is most conveniently done with a small rubber bulb placed over the end of the pipette.

Read the scale at the point at which the color of the unknown matches that of the wedge.

Calculation.—If the blood in the standard is so diluted as to make the factor $\frac{100}{Y\text{-}X} \times$ A equal 2, the hemoglobin corresponding to any reading R on the scale is 2(R-X) per cent or $2(R\text{-}X) \times \frac{15.6}{100}$ grams per 100 c.c. of blood.

Precautions.—The colorimeter cup must be kept clean by rubbing the inside surfaces with a small moist cotton swab. Even a small amount of acid hematin adhering to the sides will in time make a large error in the readings.

The wedge holding the standard must be shaken daily to keep the acid hematin in suspension. It is best to place the instrument on side when not in use.

The red cell pipette used for the diluting must be accurately graduated. A large percentage of the pipettes on the open market are inaccurate.

REFERENCES

[1]VanSlyke, D. D.: Jour. Biol. Chem., Jan., 1918, xxxiii, 31.
VanSlyke, D. D. and Stadie, W. C.: Jour. Biol. Chem., Nov., 1921, xlix, 1.
[2]Haden, R. L.: Jour. Am. Med. Assn., Oct. 28, 1922, lxxix, 1496.

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EDITORIALS

Incompatible Blood and Eclampsia

TO the various hypotheses which have from time to time been offered to explain eclampsia we may now add that of incompatibility between the blood of the mother and that of the fetus. McQuarrie (Bull. Johns Hopkins Hosp., Feb., 1923) in a careful analysis of results, suggests this as a possibility, while emphasizing that the work so far is not conclusive.

Flexner, Pearce and others have observed that agglutinated red blood cells may form hyaline thrombi in the capillaries, thereby inducing focal necrosis in the liver and occasionally also in the kidneys and elsewhere. The reaction in the liver usually is found around the portal vessels. Thrombosis precedes necrosis and is accompanied by intense engorgement. Hemorrhage follows, and typical necrosis develops within twenty-four to forty-eight hours of the injection of hemoagglutinative serum. The process appears to be chiefly mechanical, and the pathologic lesions resemble very much those observed in eclampsia.

Although the blood group, usually, is not determined by the time of birth, McQuarrie has studied a large number of bloods obtained from the umbilical cord and simultaneously from the mother, and finds that of the entire series

24.4 per cent had developed interagglutination at birth. Seventy per cent of all the cases of toxemia were comprised within this 24 per cent who had developed interagglutination. Sixty-four per cent of cases showing interagglutination manifested signs of toxemia, while only 9 per cent of cases without interagglutination showed toxemic symptoms. Of the total number of toxic cases, 93.3 per cent showed incompatibility between the maternal and fetal blood, while only 6.7 per cent of toxic cases showed true compatibility. Toxemia was found to be 16.5 times more likely to occur when the maternal and fetal bloods were incompatible than when they were of the same blood group.

McQuarrie points out that before a definite relationship between agglutination and eclampsia can be assumed it must be shown (1) that the isoagglutination characteristics of the fetus be developed at the time the toxemia appears; (2) that the fetal blood grouping differ from that of the mother; (3) that a break in the placental barrier between the two circulations occur at some point, and (4) that sufficient fetal blood enter the maternal circulation to give rise to the clinical and pathological changes observed.

In a certain number of cases, the blood group has probably been developed within the fetus within the last quarter of pregnancy and this fetal group frequently differs from that of the mother. McQuarrie's work is correlated with the observations of Young, who found that the toxemia of pregnancy is associated with premature separation of the placenta or with placental infarction with subsequent absorption of toxic products. If this be true, it may be possible to hypothecate a break in the placental barrier between the two circulations, and a fetal origin of agglutinated red cells in the maternal liver.

The total volume of blood in the fetus does not exceed 300 to 500 c.c. but it has been pointed out by Ottenberg that a few large clumps of foreign cells cause a more serious reaction than a much larger number of cells in small clumps. McQuarrie estimates that as little as 25 to 50 c.c. of fetal blood might be sufficient to cause severe reaction in the mother.

The author recognizes that his observations, while thoroughly interesting and suggestive, are not conclusive, particularly because there are unexplained exceptions in each of the various classifications of his work, and he outlines further study which may cast additional light upon the subject. The hypothesis is at any rate, attractive.

—*W. T. V.*

Achlorhydria

THE greater ease and accuracy of diagnosis with the opaque-meal in gastric pathology as compared with test-meal examination has resulted in a disposition to undervalue information obtainable from gastric analysis. On the contrary, the correlation of roentgen and test-meal findings has enhanced the value of the latter and in certain conditions has enabled us to draw more nearly correct conclusions from gastric analysis. We no longer insist that

achlorhydria must be present before a diagnosis of carcinoma of the stomach is made. No more do we insist upon the presence of hyperchlorhydria in ulcer of the stomach or duodenum. Nevertheless, the findings as made in each case of organic abdominal disease are usually of some value in summing up the diagnostic conclusions.

Increasing interest is developing in conditions associated with achlorhydria, and as more gastric analyses are being performed, achlorhydria is found to be decidedly more prevalent than was thought formerly to be the case. Hurst remarks (Lancet, Jan. 20, 1923) that out of 325 unselected cases, achlorhydria was present in 10 per cent. In an additional 9.2 per cent no free acid was found one hour after the test meal, although it was present at other times when the fractional method was employed. The frequent association between achlorhydria and pernicious anemia is well known. Various clinicians have observed that the gastric abnormality is even more constant than is the anemia itself. Achylia has repeatedly been noted, preceding sometimes by years the development of the primary anemia. That achlorhydria in these cases is possibly an hereditary affair is suggested by the finding of achylia in immediate relatives of individuals suffering from pernicious anemia.

In general two schools of thought exist in regard to the cause of this gastric secretory anomaly. There are those who maintain that the disappearance of free hydrochloric acid is secondary to a chronic gastritis. The second school maintains that the absence of acid is primary and that gastritis results only secondarily, from attempts to overwork a constitutionally inferior organ. This is borne out by the observation that in nearly all cases of achlorhydria some acid is present as combined acid, but not in sufficient amounts to become free acid. Furthermore, in such cases, operated upon for some extraneous condition, the gastric mucosa does not appear atrophied and on microscopic examination, the secretory elements are normally present, a phenomenon which does not hold in chronic gastritis. The secretory elements are histologically intact, but functionally inadequate. Finally, under proper treatment, free hydrochloric acid sometimes returns in the achlorhydria of chronic gastritis, such as alcoholic gastritis. This is not true of constitutional achlorhydria. The therapeutic deduction made by Hurst is that individuals suffering from achlorhydria should be furnished with adequate facilities for better digestion so that a chronic gastritis may not develop from constant irritation. Rational treatment consists in the administration of hydrochloric acid, a diet which will not remain too long in the stomach, and thorough mastication of the food.

Hurst lays great emphasis on the relationship between achlorhydria and infection in the mouth and upper respiratory passages. Pus and bacteria entering an anacid stomach are not digested and destroyed in the usual manner, but pass through into the intestines where they may be more readily absorbed and produce further disorder. He is inclined to attribute a certain proportion of appendicitis associated with focal infection to escape past an inefficient barrier rather than absorption directly into the blood stream. He observes that achlorhydria is not infrequently present in chronic arthritis and

that rational treatment of this disease will include local treatment of the stomach to prevent the passage of infectious agents into the intestines. That this is not always the case or that it is not the case in the majority of cases of arthritis, may be shown by the performance of gastric analysis on a series of individuals suffering from this disease.

The value of gastric analysis has increased since the institution of the fractional method of study. The discomfort to the patient and the fact that the results usually are of secondary diagnostic interest, have caused many to dispense with this valuable procedure. Like so many other items of the routine examination, it fails to give, in the majority of instances, any valuable information. But the value of routine examination of any form whatsoever lies in its tendency to bring to light the unsuspected finding in the occasional case. —*W. T. V.*

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*Ambroise Paré**

THERE is renewed interest in the life and work of the great surgeon of the sixteenth century, Ambroise Paré, and Doctor Packard has given us full information, most attractive in literary form, while the publisher has spared no pains in either text or illustration. Paré is a very striking figure. He was physician to four kings successively. There is still dispute as to whether he was Catholic or Huguenot. Evidently he practiced his profession without regard to the religion of his clientele and, although he lived in the midst of the great Protestant massacre, his historian is unable to say with certainty what his religion was. Possibly he did not have any. We are inclined after reading this charming story, to come to the conclusion that the distinguished surgeon of the sixteenth century was both wise and wily. Of lowly birth he was honored beyond any one else in his profession of the generation in which he lived. Beginning as a barber-surgeon he became the greatest man in the College of Surgeons in France and was able to hold his own even with the dominating, aristocratic, theorizing members of the College of Physicians. Brought up in poverty he became the possessor of many houses in Paris and a comfortable country home; indeed, his wealth enabled him to play the rôle of a philanthropist not only to his poor relations, but to other deserving people. His writings are at the same time punctuated with egotism and modesty. On the same page he tells us of some great cure he wrought where others had failed, and then adds: "I dressed his wounds; God healed them." His record, at least in places, sparkles with wit and elsewhere bares gems of wisdom. Above all, as an author, he is both entertaining and instructive, and this is a combination possessed by but few writers. Running through even the most important of his discoveries there is in the narrative always a personal flavor. When the College of Physicians attacked him, one of the charges was that he was ignorant of

*The Life and Times of Ambroise Paré, by Francis R. Packard, M.D., Paul B. Hoeber, New York, 1921.

Latin. To this he pleaded guilty, and one of the things which has made him great is that he had the courage to write in the vernacular. He expressed no shame on his admitting his ignorance of Latin and Greek, but pointed to the fact that he was able to hire translators. Paré made most of his reputation as an army surgeon. He was almost constantly in the field with some besieging army or in some besieged city. Great military leaders strove one with the other to secure his services and he naively tells us that after he had been smuggled into the besieged City of Metz in 1552 the dukes, princes, and other high officials in the city embraced him, supplied him with pipes of wine, and assured him that since he had arrived they would no longer fear grave disaster from the barbs of the enemy; indeed, Paré's "Journey to Metz in 1552" is a most charming story, quite equaled though by several others. This siege was most interesting from many standpoints. It was soon after the introduction of firearms in warfare. In the besieged city were nearly all, or at least a large number, of the heads of the aristocratic families of France, with about 6,000 men, all under the command of the Duke of Guise. On the outside and besieging the city was an army of 120,000 under the Duke of Alva. Guise, wise beyond his generation and apparently acquainted with some of the disasters which had previously befallen beleaguered cities, gave marked attention to the cleanliness of the city, to the storing away of vegetables for the winter, possibly with the thought of scurvy in mind, and to the health of his soldiers and of the citizens. The Duke of Alva swore by his beard that he would capture all these French notables trapped within the walls of Metz and that he would demand for each a princely ransom. Paré was smuggled into the city and, as we have seen, brought with him much cheer and comfort. After sixty-three days the plague having appeared in the besieging army, the Duke of Alva departed with his great hosts, leaving behind many sick and wounded.

It has been stated that Paré was the first to use the ligature to check hemorrhage, especially in amputations. This is not true. Paré himself made no such claim; indeed, when he was attacked for recommending ligatures, he retorted that this method of checking hemorrhage had been recommended by Hippocrates, Galen, Avicenna, John of Vigo, and others. John of Vigo, who preceded Paré about one generation taught that wounds inflicted by firearms are poisonous and recommended that all such wounds should be treated with boiling oil. In his first campaign Paré made the great discovery that this was not necessary. He tells that being compelled to treat a great number of gunshot wounds, following John of Vigo, he applied boiling oil to many, but his supply of oil being exhausted he treated the others by placing on the wounds an emulsion made of the yolks of eggs, oil of roses, and turpentine. "That night I could not sleep at my ease fearing that by lack of cauterization I would find the wounded upon which I had not used the said oil dead from the poison. I raised myself very early to visit them, when beyond my hope I found those to whom I had applied the digestive medicament feeling but little pain, their wounds neither swollen nor inflamed, and having slept through the night. The others to whom I

had applied the boiling oil were feverish, with much pain and swelling about their wounds. Then I determined never again to burn thus so cruelly the poor wounded by arquebuses."

The possibility of infection being disseminated by flies apparently struck Paré. He tells that after the Battle of San Quentin (1557) he visited the battle-field and there found many dead men and horses, with a fearful stench arising from the decomposing bodies. He says that these bodies were covered with a great number of big flies which had procreated themselves from the humidity of the dead bodies. They had tails of green and blue and when disturbed they flew in swarms so great that they made a shadow in the sun. He says: "We heard them buzzing with great wonder; and I believe that there where they settled it would render the air pestilent and cause the plague."

—*V. C. V.*

The Original Daily Dozen

CALISTHENIC exercises are enjoying an unprecedented popularity. This may be accounted for, in part at least, by the deed of some good or evil genius who has had the exercises set to music for victrola accompaniments. That setting up exercises are of ancient evolutionary origin may be suspected by observing the stretchings and yawnings of the family dog or cat after a postprandial nap.

Dinguizli has analyzed the Mohammedan prayers and their relationship to general hygiene (Bull. Acad. Med., Paris, Jan. 30, 1923). He suggests that the salaams and obeisances were incorporated in the ritual because their originator recognized the value of periodic exercise of the body. The Mohammedan prayer is at the same time a religious symbol and a cleansing or purification of the body. The true believer makes five prayers daily—the first between sundown and the rising of the sun; the second at noon, when the shadow begins to commence to grow; the third, before the setting of the sun; the fourth, during twilight, and the fifth at some hour of the night.

The faithful who at each prayer is holding an audience with his Master, is not prepared for such communion until he has rigorously followed certain procedures for the cleansing of the body. The degree of cleansing depends upon the nature of the impurities with which one may have been in contact. The greatest impurity is the accomplishment of the act of procreation. Lesser impurities result from sleep, micturition, defecation, vomiting, loss of blood, etc. The great impurity may be effaced only by bathing the entire body. Even the dirt must be removed from beneath the nails. The lesser impurities may be cleansed by less rigorous methods, such as triple washing of the hands, triple washing of the mouth, cleansing of the teeth, irrigation of the nose, etc. The cleanliness of the water and the locality where the ablutions are performed are also definitely prescribed.

At prayer, the faithful, standing erect, faces the direction of Mecca, directs his hands heavenward and pronounces: *Allah Akbar,* God is great!

He then lowers his arms or crosses them, kneels, recites the first chapter of the Koran and makes a profound reverence, bending forward to the ground. Raising his body he repeats his conviction that God is great and prostrating himself again, he gives himself entirely into the keeping of the Almighty. This is the great moment of prayer. Now the forehead and the nose must touch the ground, a symbol of the nothingness of the man,—dust before the Great Presence. Once again the trunk is elevated, and the legs are crossed beneath the body so that the anterior surfaces of the tibiae and the dorsum of each foot are in contact with the earth. Again the suppliant prostrates himself and recites formulas of glorification. Finally he arises.

This gymnastic procedure must be executed twice in the morning, four times at noon, four times in the afternoon, three times at sunset and four times during the night. There are supplementary genuflections which bring the total to about thirty daily. Cleanliness and suppleness of body are thus developed along with religion in the orthodox Mussulman. Further, the repetition of various prayers exercises the memory as well as the body.

We may assume that Mohammed like Moses was a great hygienist. In all probability neither prophet developed in their entirety the laws attributed to them, but their names stand rather as symbols for the great thinkers of their times. The true believer among the Mohammedans has taken his daily dozen for centuries, and except where infectious disease has decimated his ranks, the results, so they tell us are apparent. Dinguizli remarks on the infrequency of appendicitis in Mussulmans and suggests that it may be due in part at least to the constant exercise of the trunk muscles.

Unfortunately, in Christian and Mohammedan countries alike most of us do not follow even the simple formulas prescribed by the greatest of the ancient hygienists. If we would but follow their teachings the world would be a cleaner and healthier locality.

—*W. T. V.*

Epidemic Yaws in the Philippines

IN THE summer of 1922, Surgeon General Ireland appointed a Medical Research Board, of which Colonel George R. Callender is chairman, and sent it to Manila. The United States has a right to be proud of the scientific and medical work done in the Philippines since our occupation. Certain army medical officers have seized the opportunities presented in this new tropical field and have made most valuable contributions to our knowledge of the bacteriology and epidemiology of certain diseases there prevalent. Under the guidance of Dr. Paul Freer, the Bureau of Science in the Philippines came to be one of the most important world centers for the study of tropical diseases. Under more recent administrations this institute has apparently been allowed to lapse into desuetude. We hope it will be revived, but if this does not occur we are sure that the Medical Research Board of the Army, referred to above, will make valuable contributions to medical science.

Under date of October 30, 1922, Colonel Callender informs us that there is only four miles out of Manila an epidemic focus of yaws, with about 1,000 cases within an area of approximately four square miles or less. The Board is now able to obtain arsphenamine at the low price of 25c per tube, and within a short time we will undoubtedly have more decisive evidence concerning the curative properties of this preparation in the treatment of yaws.

The Board, in conjunction with Dr. Albert, is also studying infantile beriberi, which has been a large factor in the infantile death rate in Manila. We congratulate the Surgeon General on his wisdom in appointing the Medical Research Board for the Philippines and for selecting as its director Colonel Callender. We may rest assured that no opportunity to add to our knowledge of the diseases prevalent in the Philippines will escape this Commission. —*V. C. V.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, St. Elizabeth's Hospital, Richmond, Va.)

*Hawk's Physiological Chemistry**

THE eighth edition of any book requires no introduction. Because of the rapid development of new methods in the field of physiologic chemistry, a book on this subject which is not frequently revised becomes out of date in a remarkably short time. The eighth edition of Practical Physiological Chemistry receives the same general treatment that has characterized previous editions. The chemistry and biochemistry of enzymes, of food elements, of secretions, and the various body tissues are discussed in detail. Illustrative laboratory tests are included under each subject together with discussion of the practical application of the known facts to clinical laboratory study. This book accomplishes what few others have succeeded in doing; it is at the same time a laboratory guide for the undergraduate student and an excellent reference book for the experienced worker.

The newer practical methods of analysis are incorporated and brought up-to-date. This is seen particularly in the chapters on blood analysis and gastric analysis. A division devoted to respiration and acidosis contains highly instructive information. In the chapter on metabolism, considerable attention is devoted to the biochemistry of deficiency diseases. Two new tests are included in an addendum. These are quantitative tests for sugar and albumin in the urine, as devised by Benedict, Folin and Hawk, for routine insurance work.

The book deserves the popularity which has been accorded it in the past.

Books Received

THE MICROSCOPICAL EXAMINATION OF FOODS AND DRUGS. By Henry G. Greenish, F.I.C., F. L. S. Professor of Pharmaceutics to the Pharmaceutical Society of Great Britain and in the University of London; Director of the Pharmacy Research Laboratory; Docteur (H. C.) de l'Université de Paris; Hanbury Gold Medallist, 1917; Joint Editor of the British Pharmacopeia, 1914; Late Member of the Board of Examiners of the Pharmaceutical Society of Great Britain; Late External Examiner in Materia Medica and Pharmacy to the University of Birmingham. A Practical Introduction to the Methods adopted in the Microscopical examination of Foods and Drugs, in the entire, crushed and powdered states. Third edition. Cloth. Price $4.50. Pp. 386 with 209 illustrations. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

*Practical Physiological Chemistry. By Philip B. Hawk, M.S., Ph.D., Professor of Physiological Chemistry and Toxicology in the Jefferson Medical College of Philadelphia. With two full-page plates of absorption spectra in colors. Four additional full-page color plates and one hundred and ninety-seven figures of which twelve are in colors. A book designed for use in courses of Practical Physiological Chemistry in Schools of Medicine and of Science. Eighth edition, revised. Cloth. Price $5.00. Pp. 693. Philadelphia: P. Blakiston's Son & Co., 1012 Walnut Street. 1923.

A MANUAL OF PHARMACOLOGY and its applications to Therapeutics and Toxicology. By Torald Sollmann, M.D. Professor of Pharmacology and Materia Medica in the School of Medicine of Western Reserve University, Cleveland. Second Edition, entirely reset. Cloth. Price $5.50. Pp. 1006. Philadelphia and London: W. B. Saunders Company. 1922.

GENERAL BACTERIOLOGY. A textbook by Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago and in the Rush Medical College. Seventh Edition. Thoroughly revised. Cloth. Price $5.00. Pp. 744. Fully illustrated. Philadelphia and London. W. B. Saunders Company. 1922.

CLINICAL MEDICINE. I. TUESDAY CLINICS AT THE JOHNS HOPKINS HOSPITAL. By Lewellys F. Barker, M.D., LL.D., Professor of Medicine, Emeritus, Johns Hopkins University; Visiting Physician to Johns Hopkins Hospital, Baltimore. Cloth. Price $7.00. Pp. 617. Illustrated. Philadelphia and London. W. B. Saunders Company. 1922.

THE CHEMISTRY OF TUBERCULOSIS. By H. Gideon Wells, M.D., Ph.D., Director of the Otho S. A. Sprague Memorial Institute, Professor of Pathology in the University of Chicago and in Rush Medical College; Lydia M. DeWitt, M.D., A.M., Member of the Otho S. A. Sprague Memorial Institute, Associate Professor of Pathology in the University of Chicago and in Rush Medical College; Esmond R. Long, Ph.D., Assistant Professor of Pathology in the University of Chicago and in Rush Medical College. Cloth, price $5.00. Pp. 438. Baltimore, Williams and Wilkins Company.